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43

Annals of Surgery

43

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Cefotan arithmetic.

+ more cost-effective than cefoxitin[1,2]

− less frequent dosing than cefoxitin[3,4]

× several times the half-life of cefoxitin[5,6]

÷ single-dose prophylaxis/ twice-daily treatment

P. 24,812

Count on
CEFOTAN® IM/IV
(cefotetan disodium)
In intra-abdominal and gynecologic infection due to indicated organisms

References: 1. Nightingale CH, Smith KS, Quintiliani R, et al: The conversion of cefoxitin usage to cefotetan: An interdisciplinary approach. *Am J Surg* 1988;155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT: Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference*, ed 43. Oradell, NJ, Medical Economics Co, 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH: Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg*, April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, et al: Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg* 1988;155(5A):67-70.

Please see adjacent page for brief summary of prescribing information.

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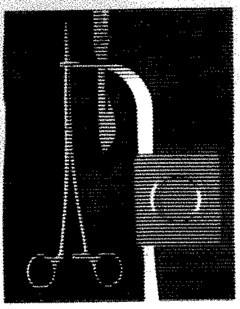

Count on CEFOTAN® IM/IV (cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE

TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.
NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS

GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.
INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.
DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.
CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be

CEFOTAN® (cefotetan disodium)

developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.
USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.
USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.
PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION

TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.
IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function:

$$\text{Males:}\quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:}\quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED

CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

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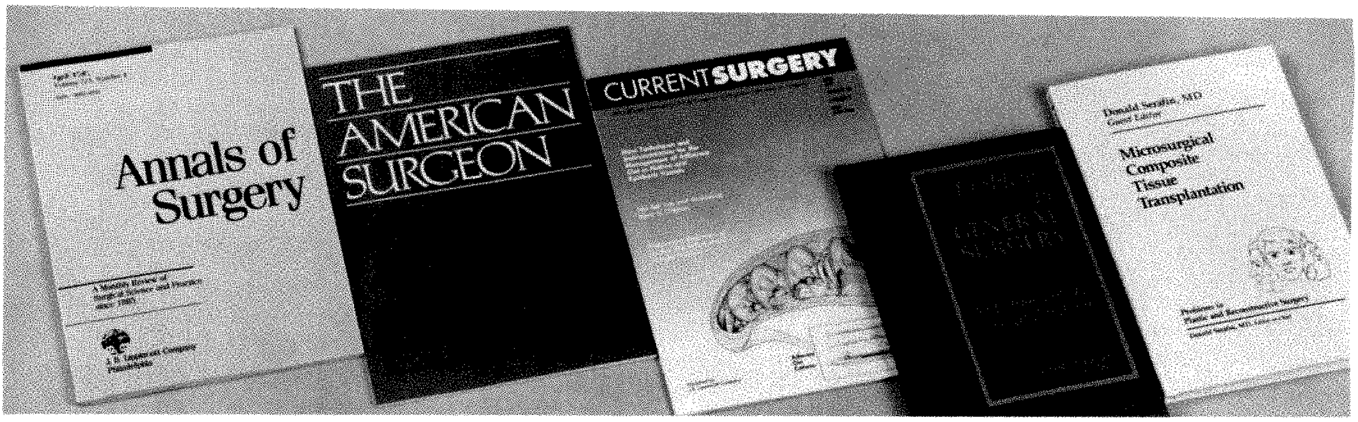

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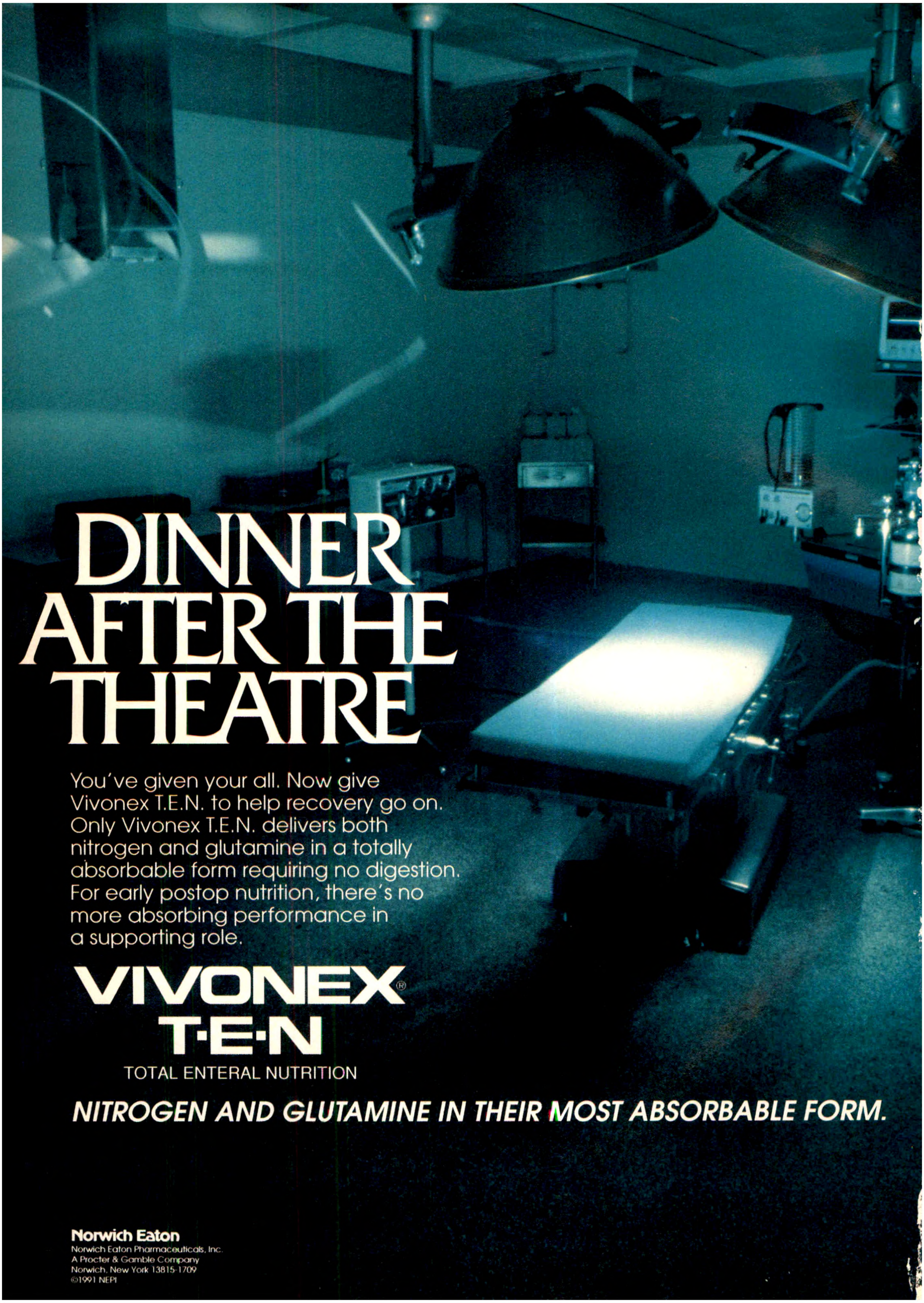

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Annals of Surgery

CONTENTS

EDITORIAL

Retroperitoneal Sarcoma 1
LaSalle D. Leffall, Jr., M.D.
Washington, D.C.

WHAT'S NEW IN GENERAL SURGERY

Diagnosis and Management of Retroperitoneal Soft-tissue Sarcoma 2

The application of systematic diagnostic and biopsy procedures and an aggressive operative approach can result in complete resection and significantly improved overall survival in 50% of patients with retroperitoneal sarcoma. However, despite removal of all gross tumor, 90% of patients develop and die of local recurrence. A National Retroperitoneal Sarcoma Registry and cooperative intergroup studies are urgently needed to develop innovative combined modality strategies to alter this pattern of local failure.

F. Kristian Storm, M.D., and David M. Mahvi, M.D.
Madison, Wisconsin

ORIGINAL ARTICLES

Verification of a New Clinicopathologic Staging System for Colorectal Adenocarcinoma 11

A new clinicopathologic staging system for colorectal adenocarcinoma is proposed. It incorporates stage, race, tumor morphology, and microinvasion. The new staging system predicts 5-year survival more accurately than Dukes' staging alone for both colon and rectal adenocarcinoma.

Fabrizio Michelassi, M.D., F.A.C.S., Jaime J. Ayala, M.D., Tommaso Balestracci, M.D., Ruth Goldberg, M.D., Richard Chappell, and George E. Block, M.D., F.A.C.S.
Chicago, Illinois

A Comparison of Intensive Care Unit Care of Surgical Patients in Teaching and Nonteaching Hospitals 19

A survey of surgical intensive care units at teaching and nonteaching hospitals is presented. The manner in which care is delivered and the views of the respondents are analyzed. A discrepancy between what is desired and what is actually practiced exists at both types of hospitals.

Samir M. Fakhry, M.D., Jeffrey L. Buehrer, M.D., George F. Sheldon, M.D., and Anthony A. Meyer, M.D., PH.D.
Chapel Hill, North Carolina

Differential Pathophysiology of Bacterial Translocation After Thermal Injury and Sepsis 24

Although the transient bacterial translocation that occurs following uncomplicated thermal injury appears to result from an intestinal ischemic injury, chronic gut barrier failure associated with sepsis after injury occurs independently of continued intestinal ischemia.

William G. Jones II, M.D., Annabel E. Barber, M.D., Joseph P. Minei, M.D., Thomas J. Fahey III, M.D., G. Tom Shires III, M.D., and G. Tom Shires, M.D.
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July 1991

Vol. 214, No. 1

CONTENTS

Continued

Management and Long-term Outcome of Aortic Dissection **31**

A single institutional experience of all 163 patients admitted with aortic dissection from 1975 to 1988 was reviewed. Although the hospital mortality rate was favorable, significant long-term mortality rates from concurrent cardiovascular disease and residual aortic disease mandate careful, prolonged follow-up.

Donald D. Glower, M.D., *Rudiger H. Speier,* M.D., *William D. White,* M.P.H., *L. Richard Smith,* M.D.,
J. Scott Rankin, M.D., *and Walter G. Wolfe,* M.D.
Durham, North Carolina

Ten-year Experience with Cyclosporine as Primary Immunosuppression in Recipients of Renal Allografts **42**

An experience using cyclosporine as primary immunosuppression in renal transplant recipients during a 10-year period is reported. Early overall results are satisfactory; however there is a progressive and continuing attrition rate of cadaver grafts after several years of function.

Nicholas L. Tilney, M.D., *Amy Chang,* R.N., *Edgar L. Milford,* M.D., *W. David Whitley,* M.D.,
J. Michael Lazarus, M.D., *Eleanor L. Ramos,* M.D., *Terry B. Strom,* M.D., *Charles B. Carpenter,* M.D.,
and Robert L. Kirkman, M.D.
Boston, Massachusetts

Delayed Presentation and Treatment of Popliteal Artery Embolism **50**

Recent experience with embolism to the popliteal artery demonstrated that as many as one third of these patients may present in delayed fashion. Complete angiography, an appropriate index of suspicion, and embolectomy performed through a direct popliteal approach are the essential factors in successful management.

Richard P. Cambria, M.D., *Brian A. Ridge,* M.D., *David C. Brewster,* M.D., *Ashby C. Moncure,* M.D.,
R. Clement Darling, M.D., *and William M. Abbott,* M.D.
Boston, Massachusetts

Plasma Gastrin and Cholecystokinin Response After Pylorus-preserving Pancreatoduodenectomy with Billroth-I Type of Reconstruction **56**

Plasma gastrin and cholecystokinin were measured after pylorus-preserved pancreatoduodenectomy (PPPD), standard PD-(sPD), and in a control group. Postprandial plasma gastrin and CCK responses in PPPD patients, but not sPD patients, resembled that of the control group.

Akira Tangoku, M.D., *Masahiro Nishikawa,* M.D., *Atsushi Adachi,* M.D., *and Takashi Suzuki,* M.D.
Ube, Japan

Acute Appendicitis Following Blunt Abdominal Trauma: Incidence or Coincidence? **61**

This paper describes two patients who developed appendicitis after blunt abdominal trauma. The authors discuss the possible association between these two entities and highlight the need to consider appendicitis in patients with lower abdominal pain following blunt trauma.

Mark H. Hennington, M.D., *Ellis A. Tinsley, Jr.,* M.D., *Herbert J. Proctor,* M.D., *and*
Christopher C. Baker, M.D.
Chapel Hill, North Carolina

Small-volume Hypertonic Saline Dextran Resuscitation from Canine Endotoxin Shock **64**

Lactated Ringer's solution alone and an initial bolus of hypertonic saline dextran followed by lactated Ringer's solution were equally effective in resuscitating dogs in endotoxin shock. The total fluid requirements and the net fluid gain were significantly less in dogs whose resuscitation was initiated with hypertonic saline.

Jureta W. Horton, PH.D., *and Paula B. Walker,* B.S.
Dallas, Texas

July 1991 Vol. 214, No. 1

CONTENTS

Continued

Composition of Fat in Enteral Diets Can Influence Outcome in Experimental Peritonitis 74

The role of both the amount and the type of fat used in enteral diets fed to guinea pigs with peritonitis was explored. Although the amount of fat did not strongly influence outcome, survival rates were improved by using diets with equal amounts of omega-6 and omega-3 fatty acids.

Michael D. Peck, M.D., SC.D., *Cora K. Ogle,* PH.D., *and J. Wesley Alexander,* M.D., SC.D.
Cincinnati, Ohio

Anti-tumor Effect of L-methionine-Deprived Total Parenteral Nutrition with 5-Fluorouracil Administration on Yoshida Sarcoma-bearing Rats 83

Methionine depletion induced by total parenteral nutrition infusing the methionine- and cysteine-lacking amino acid solution as a sole nitrogen source showed a synergetic effect on 5-fluorouracil. By this treatment, the hematogenous metastasis was lessened and apparent elongation of life span was obtained in a Yoshida sarcoma-bearing rat experiment.

Narihide Goseki, M.D., *Mitsuo Endo,* M.D., *Tokio Onodera,* M.D., *and Goro Kosaki,* M.D.
Tokyo, Japan

Book Reviews 89

Notice to Contributors and Subscribers 92

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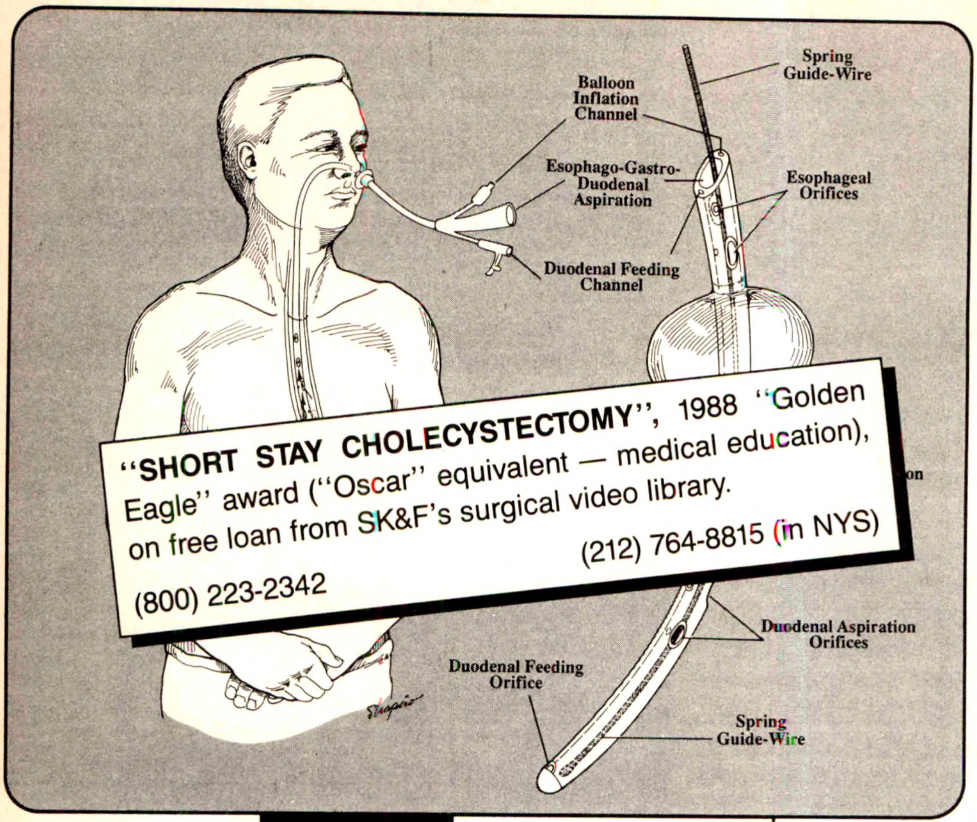

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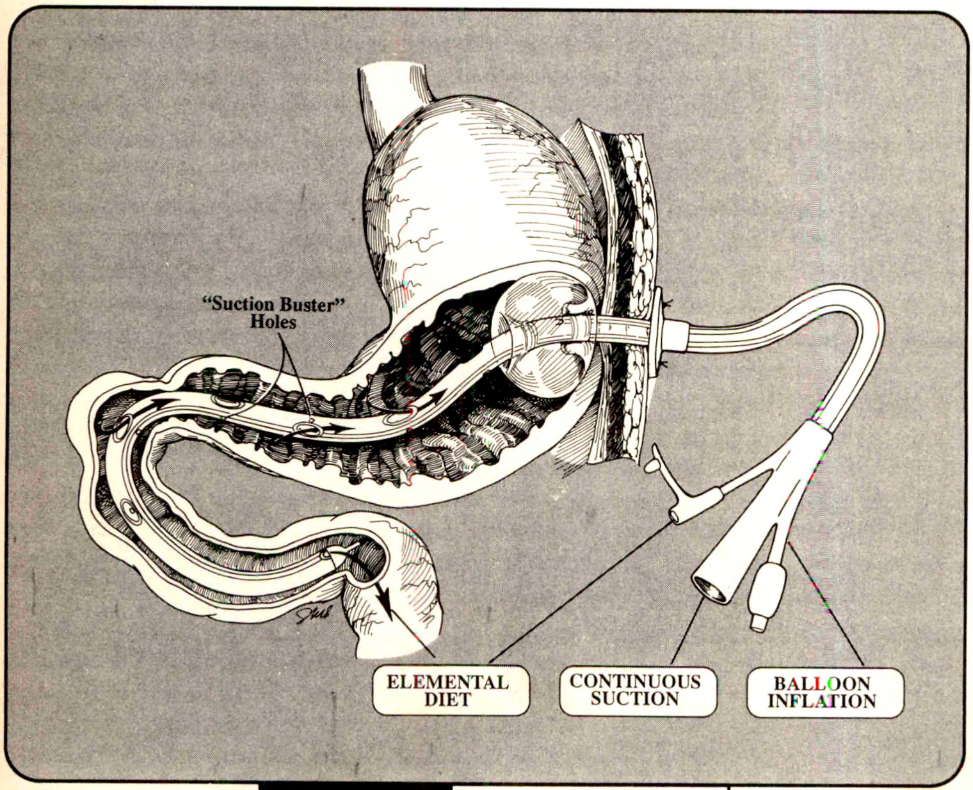

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August 1991

Vol. 214 No. 2

Annals of Surgery

CONTENTS

EDITORIAL

Rejection Revisited 97

R. Randal Bollinger, M.D.
Durham, North Carolina

WHAT'S NEW IN GENERAL SURGERY

The Biology of Acute Transplant Rejection 98

The phenomenon of acute rejection of an allograft involves a series of complex and inter-related cellular and humoral events, culminating in graft death.

Nicholas L. Tilney, M.D., *and Jerzy W. Kupiec-Weglinski,* PH.D., M.D.
Boston, Massachusetts

ORIGINAL ARTICLES

The Influence of Intraoperative Hypotension and Perioperative Blood Transfusion on Disease-free Survival in Patients with Complete Resection of Colorectal Liver Metastases . . 107

Multivariate analysis of clinical and perioperative prognostic factors that influence the outcome of patients with completely resected colorectal metastases suggests that episodes of intraoperative hypotension adversely affect disease-free survival.

Riad N. Younes, M.D., *Andre Rogatko,* PH.D., *and Murray F. Brennan,* M.D.
New York, New York

Intrahepatic Recurrence After Resection of Hepatocellular Carcinoma Complicating Cirrhosis 114

Forty-seven patients with hepatocellular carcinoma complicating cirrhosis underwent a curative resection. The main result of this study is that resection is almost ineluctably followed by intrahepatic recurrence, even when apparently satisfactory (single tumor, extension carefully assessed by preoperative ultrafluid lipiodol and intraoperative ultrasound).

Jacques Belghiti, Yves Panis, Olivier Farges, Jean Pierre Benhamou, and Francois Fekete
Paris, France

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CONTENTS

Continued

Radiolabeled Antibody Imaging in the Management of Colorectal Cancer: Results of a Multicenter Clinical Study **118**

Results of a multi-institutional trial are reported on the usefulness of ^{111}In CYT-103 radiolabeled monoclonal antibody in 116 presurgical colorectal cancer patients. The contribution of immunoscintigraphy to surgical decision making through the detection of occult disease and confirmation of localized potentially resectable disease is outlined.

Ralph J. Doerr, M.D., Hani Abdel-Nabi, M.D., David Krag, M.D., and Edith Mitchell, M.D.
Buffalo, New York; Davis, California; and Columbia, Missouri

The Prognosis of Melanoma Patients with Metastases to Two or More Lymph Node Areas **125**

Twenty-one melanoma patients presented with or later developed metastatic involvement of two or more noncontiguous lymph node regions. The prognosis of these patients, after therapeutic lymphadenectomy, appeared to be equivalent to that of patients with metastatic involvement of only one regional node site.

Richard J. Barth, Jr., M.D., David J. Venzon, PH.D., and Alan R. Baker, M.D.
Bethesda, Maryland

Metabolic Consequences of (Regional) Total Pancreatectomy **131**

Forty-nine patients were studied. Anorexia and diarrhea were present early, but abated spontaneously. Weight was gained or maintained. Diabetic instability was constant. Over the long term, there was an unusual frequency of liver disease and osteopenia. Overt diabetic micro- and macrovascular disease was absent. Performance was good in long-term survivors.

Carolyn M. Dresler, M.D., Joseph G. Fortner, M.D., Katherine McDermott, R.N., and Daiva R. Bajorunas, M.D.
New York, New York

The Complex Pattern of Cytokines in Sepsis: Association Between Prostaglandins, Cachectin, and Interleukins **141**

Although various immunologic changes occur during sepsis, it is unknown whether there is any association between the release of cytokines (TNF, IL-1, and IL-6) and prostanoids. These results indicate that the altered macrophage prostanoid release and elevated plasma prostanoids may regulate the release and consequently the circulating levels of cytokines during sepsis.

Wolfgang Ertel, M.D., Mary H. Morrison, M.S., Ping Wang, M.D., Zheng F. Ba, B.S., Alfred Ayala, PH.D., and Irshad H. Chaudry, PH.D.
East Lansing, Michigan

Lovastatin Inhibits Gallstone Formation in the Cholesterol-fed Prairie Dog **149**

Administration of lovastatin, a drug to treat hyperlipidemia, reduced the incidence of gallstones in a diet-induced model of cholesterol gallstones. The efficacy of this agent is probably secondary to its known inhibitory effect on cholesterol biosynthesis.

Kimberly D. Saunders, M.D., Joe A. Cates, M.D., Mohammad A. Abedin, PH.D., Sheila Rege, M.D., Sarkis F. Festekdjian, B.A., Warren Howard, B.A., and Joel J. Roslyn, M.D.
Los Angeles, California

Dose Response of Fish Oil *Versus* Safflower Oil on Graft Arteriosclerosis in Rabbit Heterotopic Cardiac Allografts **155**

Dose response of fish oil on accelerated graft coronary arteriosclerosis was assessed in a rabbit heterotopic cardiac allograft model, using safflower oil as a caloric control. When the animals were killed, allograft vessel disease appeared to exhibit an unfavorable dose response to fish oil as well as safflower oil, independent of effects on plasma lipoproteins and low-density lipoprotein oxidation potential.

Kwok L. Yun, M.D., James I. Fann, M.D., Mitchell H. Sokoloff, B.A., Loren G. Fong, PH.D., George E. Sarris, M.D., Margaret E. Billingham, M.B., M.D., and D. Craig Miller, M.D.
Palo Alto, California

CONTENTS

Continued

Adverse Effect of Therapeutic Vasoconstrictors in Experimental Acute Pancreatitis **168**

Perfusion of the pancreas appears to be a critical factor in the pathogenesis of necrotizing pancreatitis. Phenylephrine increased the clinical and morphologic severity of cerulein-induced acute pancreatitis in rats. Vasoconstrictors should be used only with caution in patients with acute pancreatitis.

E. Klar, M.D., D. W. Rattner, M.D., C. Compton, M.D., G. Stanford, M.D., B. Chernow, M.D., and A. L. Warshaw, M.D.
Heidelberg, West Germany; and Boston, Massachusetts

The Local Effects of Cachectin/Tumor Necrosis Factor on Wound Healing **175**

The local effects of cachectin/tumor necrosis factor (TNF) on experimental wounds are evaluated. TNF appears to be detrimental to wound healing as it decreases bursting strength and activity of the gene for collagen.

Gary D. Salomon, M.D., Attan Kasid, PH.D., Douglas T. Cromack, M.D., Elaine Director, B.S., Thomas L. Talbot, M.S., Anthony Sank, M.D., and Jeffrey A. Norton, M.D.
Bethesda, Maryland

Interleukin-2 Induces Early Multisystem Organ Edema Mediated by Neutrophils **181**

A 1-hour infusion of interleukin-2 leads to edema of the heart and lungs, mediated largely by activated neutrophils.

Richard Welbourn, M.B., F.R.C.S., Gideon Goldman, M.D., Lester Kobzik, M.D., Ian Paterson, M.B., F.R.C.S., David Shepro, PH.D., and Herbert B. Hechtman, M.D.
Boston, Massachusetts

Acceleration of Superoxide Production from Leukocytes in Trauma Patients **187**

The inflammatory response in trauma patients is modulated by the functional capacity of neutrophils. Neutrophils collected from early-phase trauma patients demonstrated an increased production of superoxide anion. Tumor necrosis factor potentiated superoxide production and elastase release by neutrophils, and may function as a modulator of the inflammatory response.

Hiroshi Tanaka, M.D., Hiroshi Ogura, M.D., Junichiro Yokota, M.D., Hisashi Sugimoto, M.D., Toshiharu Yoshioka, M.D., and Tsuyoshi Sugimoto, M.D.
Osaka, Japan

Book Reviews **193**

Please see the July 1991 issue for the complete Notice to Contributors and Subscribers.

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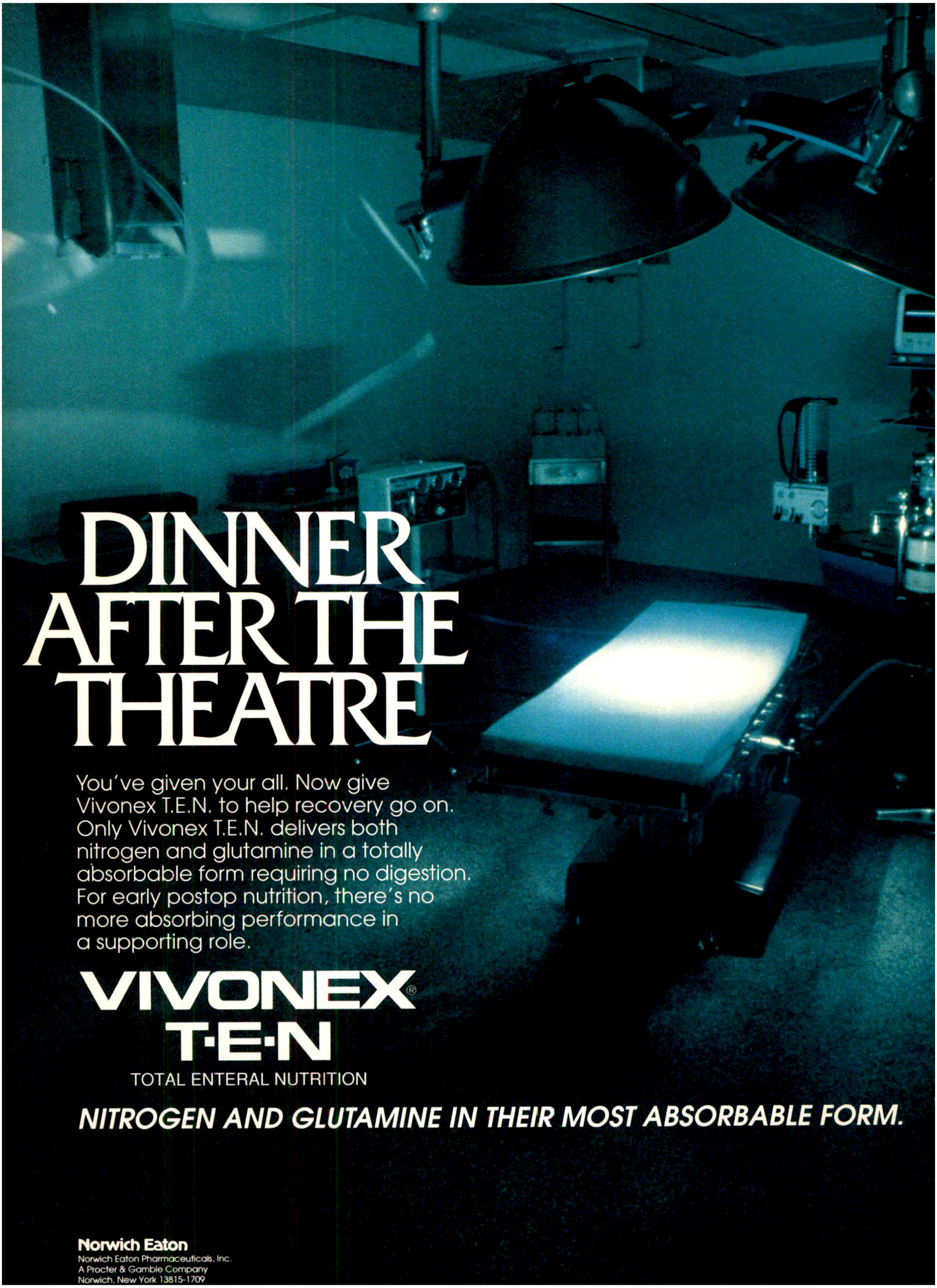

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September 1991

Vol. 214 No. 3

Annals of Surgery

CONTENTS

PRESIDENTIAL ADDRESS

The Right Thing 197
Robert Zeppa, M.D.
Miami, Florida

SCIENTIFIC PAPERS

The Optimal Extent of Resection for Patients With Stages I or II Breast Cancer Treated With Conservative Surgery and Radiotherapy 200

An extensive intraductal component (EIC) in or adjacent to an invasive ductal carcinoma of the breast strongly influences the incidence of local recurrence in the breast after local excision and radiotherapy. In 509 patients with T1 and T2 lesions, the presence of EIC significantly increased the development of local recurrence.

Frank A. Vicini, M.D., *Timothy J. Eberlein,* M.D., *James L. Connolly,* M.D., *Abram Recht,* M.D., *Anthony Abner,* M.D., *Stuart J. Schnitt,* M.D., *William Silen,* M.D., *Jay R. Harris,* M.D.
Boston, Massachusetts

NSABP Trials of Adjuvant Chemotherapy for Breast Cancer: A Further Look at the Evidence 206

Five clinical trials of adjuvant chemotherapy carried out by NSABP provide evidence that such treatment is not effective in prolonging survival in premenopausal patients with Stage II breast cancer. Recommendations that this become standard therapy should be reconsidered.

C. Barber Mueller, M.D., *and Mary L. Lesperance,* PH.D.
Hamilton, Ontario, Canada

Dissociation of Blood Volume and Flow in Regulation of Salt and Water Balance in Burn Patients 213

Decreased blood volume appears to be the major stimulus for regulation of antidiuretic hormone secretion after thermal injury. Blood flow remains elevated despite measured decrements in blood volume in the flow phase of injury.

William G. Cioffi, Jr., M.D., *George M. Vaughan,* M.D., *James D. Heironimus,* M.D., *Bryan S. Jordan,* R.N., M.S.N., *Arthur D. Mason, Jr.,* M.D., *and Basil A. Pruitt, Jr.,* M.D.
San Antonio, Texas

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September 1991 Vol. 214, No. 3

CONTENTS

Continued

Hepatic Resection *Versus* Transplantation for Hepatocellular Carcinoma **221**

The survival rate after transplantation (TX) for hepatocellular carcinoma (HCC) and that after hepatic resection (HX) were compared according to the pTNM stages. The survival rates were similar in each stage of pTNM. However, TX provided significantly better survivals than HX in each stage when HCC was associated with cirrhosis of the liver.

Shunzaburo Iwatsuki, M.D., *Thomas E. Starzl,* M.D., PH.D., *Daniel G. Sheahan,* M.B., M.SC., *Itsuo Yokoyama,* M.D., *Anthony J. Demetris,* M.D., *Saturo Todo,* M.D., *Andreas G. Tzakis,* M.D., *David H. Van Thiel,* M.D., *Brian Carr,* M.D., PH.D., *Richard Selby,* M.D., *and Juan Madariaga,* M.D.
Pittsburgh, Pennsylvania

Primary and Recurrent Crohn's Disease: Experience With 1379 Patients **230**

Crohn's disease involving multiple gastrointestinal sites is associated with two and one-half times the rate of recurrence of Crohn's disease limited to a single site. Presence of perineal disease is associated with a significantly higher risk of local recurrence. Colonic disease carries a five-fold greater risk of stoma placement than other sites.

Fabrizio Michelassi, M.D., F.A.C.S., *Tommaso Balestracci,* M.D., *Rick Chappell,* PH.D., *and George E. Block,* M.D., F.A.C.S.
Chicago, Illinois

Treatment of Chronic Traumatic Bone Wounds: Microvascular Free Tissue Transfer. A 13-Year Experience in 96 Patients **241**

Over a 13-year period 96 patients with chronic osteomyelitis were treated with microvascular free tissue transfers. The recurrence rate of osteomyelitis in this treatment group was 4.2%. Chronic traumatic osteomyelitis is a potentially curable entity when complete debridement is followed by viable soft-tissue reconstruction.

James W. May, Jr., M.D., *Jesse B. Jupiter,* M.D., *G. Gregory Gallico, III,* M.D., *Douglas M. Rothkopf,* M.D., *and Paula Zingarelli*
Boston, Massachusetts

The Role of Prostaglandin E_2 in Immune Suppression Following Injury **253**

Evidence is presented that in humans PGE_2 plays a role in the suppression of cellular immunity after burn injury by inhibition of lymphocyte IL-2 production. Endotoxin mimics the effect of injury on PGE_2 production, T-cell activation, and IL-2 production.

John T. Grbic, D.M.D., *John A. Mannick,* M.D., *David B. Gough,* F.R.C.S.I., M.CH., PH.D., *and Mary Rodrick,* PH.D.
Boston, Massachusetts

Immunomodulatory Therapy With Thymopentin and Indomethacin: Successful Restoration of Interleukin-2 Synthesis in Patients Undergoing Major Surgery **264**

In a prospective randomized study, in 60 patients (63 ± 3 yr) undergoing open heart surgery, the immunorestorative potential of a combined therapy with Thymopentin (TP-5) and the cyclooxygenase inhibitor Indomethacin was investigated. Perioperative immunologic screening included: DTH response, PBMC proliferative responses, phenotyping (CD3+, CD4+, IL-2R+), *in vitro* IL-2 synthesis, and serum levels of γ-IFN. In contrast to a single-agent therapy, combined TP-5 and Indo treatment restored the impaired receptor expression, lymphocyte proliferation, and IL-2 synthesis. Thus, simultaneous PGE_2 blockage and T-cell recruitment offers adequate T-cell protection in traumatized patients.

Eugen Faist, M.D., *Andreas Markewitz,* M.D., *Dietmar Fuchs,* M.D., *Stefan Lang,* M.S., *Susanne Zarius,* B.S., *Friedrich-Wilhelm Schildberg,* M.D., *Helmut Wachter,* M.D., *and Bruno Reichart,* M.D.
Munich, FRG, and Innsbruck, Austria

September 1991 Vol. 214, No. 3

CONTENTS

Continued

Chronic Visceral Ischemia: Three Decades of Progress **276**

Transaortic visceral endarterectomy and antegrade visceral bypass were performed in 74 patients with chronic visceral ischemia. The perioperative mortality rate was 12.2%. Sustained relief of symptoms was achieved in 86.5% and 86.1% of patients, respectively, at 5 years. Intraoperative transesophageal echocardiography and Duplex ultrasonography of the visceral reconstruction, combined with use of the transabdominal medial visceral rotation approach, have contributed to these improved results.

Christopher G. Cunningham, M.D., Linda M. Reilly, M.D., Joseph H. Rapp, M.D., Peter A. Schneider, M.D., and Ronald J. Stoney, M.D.
San Francisco, California

Preoperative Optimization of Cardiovascular Hemodynamics Improves Outcome in Peripheral Vascular Surgery: A Prospective, Randomized Clinical Trial **289**

This clinical study is a prospective, randomized trial of the use of the Swan-Ganz catheter with optimization of oxygen transport pre-, intra-, and postoperatively. A reduction in perioperative cardiac complications and improved bypass graft patency was documented.

Jon F. Berlauk, M.D., Jerome H. Abrams, M.D., Ian J. Gilmour, M.D., S. Rhiannon O'Conner, M.D., David R. Knighton, M.D., and Frank B. Cerra, M.D.
Minneapolis, Minnesota

Changing Trends in Necrotizing Enterocolitis: Experience With 302 Cases in Two Decades **300**

The survival of 302 infants with necrotizing enterocolitis (NEC) treated during 1972–1982 (N = 176) and 1983–1990 (N = 126) was compared. Despite lower birth weight, earlier gestational age, and later symptom onset, the overall survival in the 1983–1990 group improved from 58% to 82%. Early recognition and referral, prompt surgical intervention, and improved neonatal intensive care played a role in the improved survival.

Jay L. Grosfeld, M.D., Henry Cheu, M.D., Marc Schlatter, M.D., Karen W. West, M.D., and Frederick J. Rescorla, M.D.
Indianapolis, Indiana

Sixteen-year Experience With Aortic Root Replacement: Results of 172 Operations **308**

One-hundred sixty-eight patients underwent 172 aortic root replacements during a 16-year interval ending in October, 1990. The satisfactory long-term results with the composite graft technique support its continued use as the preferred method of treatment for patients with annuloaortic ectasia and aortic dissection who require aortic valve replacement. The availability of aortic allografts and the perfection of techniques for implantation of the pulmonary root into the aortic position have broadened the indications for aortic root replacement.

Nicholas T. Kouchoukos, M.D., Thomas H. Wareing, M.D., Suzan F. Murphy, R.N., and Johanna B. Perrillo, R.N.
St. Louis, Missouri

Second-look Surgery for Colorectal Cancer: The Second Time Around **321**

A comparison is made between standard surgical decision making with regard to metastatic colon cancer and surgical decision making that is influenced by a variety of techniques, including intraoperative radioimmunoguidance. The comparison suggests that an improvement in management can be accomplished when intraabdominal radioimmunoguidance is used to identify tumors that may otherwise not be recognized.

Edward W. Martin, Jr., M.D., and Larry C. Carey, M.D.
Tampa, Florida

September 1991 Vol. 214, No. 3

CONTENTS

Continued

The Role of Multimodality Therapy in Soft-tissue Sarcoma **328**

Soft-tissue sarcomas are rare and uncommon neoplasms. The recent 8-year experience with these tumors in one institution is reviewed, and the results of a prospective randomized trial of adjuvant brachytherapy in extremity lesions are reported. A decrease in local recurrence was shown, but no effect was seen on survival.

Murray F. Brennan, M.D., Ephraim S. Casper, M.D., Louis B. Harrison, M.D., Man H. Shiu, M.D.,
Jeffrey Gaynor, PH.D., and Steven I. Hajdu, M.D.
New York, New York

Transplantation of Islet Allografts and Xenografts in Totally Pancreatectomized Diabetic Dogs Using the Hybrid Artificial Pancreas **339**

The use of an islet-containing hybrid artificial pancreas to maintain normoglycemia in diabetic, pancreatectomized dogs is described. The device uses physical barrier techniques to contain the islets and to eliminate the need for immunosuppression. It has successfully supported the transplantation of both allogeneic and xenogeneic islet preparations.

Anthony P. Monaco, M.D., Takashi Maki, M.D., PH.D., Hiroko Ozato, M.D., Mauro Carretta, M.D.,
Susan J. Sullivan, PH.D., Kermit M. Borland, PH.D., Michele D. Mahoney, M.S., William L. Chick, M.D.,
Thomas E. Muller, PH.D., Jacqueline Wolfrum, PH.D., and Barry Solomon, PH.D.
Boston, Shrewsbury, and Lexington, Massachusetts

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EDITORIAL

Retroperitoneal Sarcoma

The article by Storm and Mahvi, "Diagnosis and Management of Retroperitoneal Soft-tissue Sarcoma," in this issue addresses a continuing dilemma for even the most skilled surgeon. Soft-tissue sarcomas are uncommon tumors (only 1% of all solid malignancies) and often are treated inappropriately regardless of their location. Retroperitoneal sarcomas, by virtue of their location and relative lack of symptoms, often present as large neoplasms. Of the tumors that can be resected for possible cure, the vast majority ultimately cause the patient's death.

Better diagnostic and therapeutic measures are required. However it is unlikely that improved diagnostic methods will be of great value because patients usually present with symptoms only when the tumor has attained large size. The roles of abdominal computed tomography and magnetic resonance imaging are well established to determine the presence, consistency, extent, and spatial relationships of these neoplasms. Open biopsy is recommended. As is true for other soft-tissue sarcomas, the histopathologic grade is a major determinant of the stage of disease, with higher grade tumors having a worse prognosis.

In recent years, malignant fibrous histiocytoma has become the most common soft-tissue sarcoma of adults. In most reported series to date, liposarcoma is the most common retroperitoneal sarcoma. Only time will determine if there will be a similar change in the histologic type of tumors occurring in the retroperitoneum.

One of the most encouraging and dramatic reports in oncology is the improved survival and quality of life for patients with soft-tissue sarcomas of the extremities. Limb-sparing surgery for low-grade tumors along with adjuvant radiation and chemotherapy for high-grade lesions has decreased markedly the amputation rate for these neoplasms and prolonged survival. For the less commonly occurring, larger retroperitoneal sarcomas, there are no such encouraging results.

The ideal treatment for soft-tissue sarcomas is wide surgical excision. With their proximity and attachment to and often invasion of major structures, retroperitoneal tumors often are nonresectable for cure at initial presentation. Clear margins of resection are difficult to obtain, thus decreasing chances for cure. Tumor debulking, euphemistically termed 'cytoreductive surgery,' has yielded varying results, but when purposely applied can lead to prolonged disease-free intervals and improved survival rates. Assiduous planning and excellent judgment are paramount to ensure that major organs and structures are not sacrificed needlessly when total or partial resection is attempted. Inappropriate resection of vital structures, when major tumor volume cannot be removed, should be discouraged. Often adjacent organs, such as the kidney, bowel, major blood vessels, and abdominal wall, must be resected to achieve adequate margins around the tumor. Thus an experienced team including oncologic, vascular, urologic, and plastic surgeons may be required. Recognizing that complete surgical extirpation offers the best chance for cure for these wily neoplasms, the initial operation should be planned and executed as carefully as possible.

In contrast to the benefit most patients with high-grade soft-tissue sarcomas receive from adjuvant radiation and chemotherapy, these modalities have been of little value for retroperitoneal tumors. Even when complete gross resection can be achieved, almost always with minimal margins in some areas, the overall outlook for cure is still poor. Clearly better therapeutic measures are needed. The role of other therapeutic regimens, including biologic response modifiers or neoadjuvant therapy, may help with this enigmatic problem.

The authors' recommendation for a national, or perhaps better, an international registry seems appropriate, where intergroup cooperative trials can be conducted in major centers. In the meantime, surgeons who treat these patients should be ever mindful that at present wide surgical excision with the best possible margins that can be obtained remains the hallmark in the management of these difficult tumors.

LaSalle D. Leffall, Jr., M.D.
Washington, D.C.

WHAT'S NEW IN GENERAL SURGERY

Diagnosis and Management of Retroperitoneal Soft-tissue Sarcoma

F. KRISTIAN STORM, M.D., and DAVID M. MAHVI, M.D.

From the Division of Surgical Oncology, Department of Surgery, University of Wisconsin, Madison, Wisconsin

Retroperitoneal soft-tissue sarcomas are locally invasive tumors that remain occult for long periods and grow quite large due to the abdominal cavity's remarkable ability to accommodate these slowly expanding masses with a paucity of attendant symptoms. An open biopsy is required to establish diagnosis definitively. Despite improved imaging techniques and preoperative and intraoperative patient management, resectability has not changed significantly in the past 20 years. Even with an aggressive operative approach, only one half the tumors can be resected completely, and of those, more than 90% recur locally and result in the death of the patient. The addition of adjuvant radiotherapy or chemotherapy has not altered this pattern of local failure, in contrast to promising results with extremity soft-tissue sarcoma. Because of the rarity of these tumors, there is an urgent need to establish a national retroperitoneal sarcoma registry and to form cooperative intergroup studies to evaluate, treat, and apply innovative multimodality combination therapies to these otherwise lethal tumors.

RETROPERITONEAL SOFT-TISSUE sarcomas are comprised of a heterogenous group of rare and capricious mesenchymal neoplasms. Soft-tissue sarcomas account for 1% of all solid tumors, with an incidence of approximately 2 in 100,000, or about 6000 new cases in the United States per year. Of these 15% will occur in the retroperitoneum (1000 cases per year). These tumors have a well-earned reputation for regional invasion and local recurrence and have humbled many a skilled surgeon. But, in fact, it is the surgeon who has the greatest likelihood of rendering such patients disease free, and it is for this reason that general surgeons must take the time and effort to understand fully and appreciate the biologic nature of these unusual neoplasms. While no large prospective randomized clinical trials exist, accumulative past experience clearly illustrates several important principles of management.

Presentation and Differential Diagnosis

Retroperitoneal sarcomas have a peak incidence in the fifth decade of life, although they have occurred in patients in their teens. In patients ($n = 675$)[1] with a retroperitoneal neoplasm, 82% were malignant and 18% benign. Of those with malignancy, 40% were lymphoma or some variety of urogenital cancer and 55% were sarcoma. Stated another way, nearly one half of all retroperitoneal solid neoplasms will prove to be soft-tissue sarcoma.

Patients most frequently present with a nontender palpable mass (80% to 90%). They may give a history of increasing abdominal girth and frequently (40% to 70%) describe vague poorly localized discomfort due to stretching of the peritonea. One third of patients will have some distal neurologic sign or symptom from the mass effect, stretching or compressing the lumbar or pelvic nerve plexes. Less commonly nonmalignant serous ascites due to external portal vein obstruction, and gastrointestinal symptoms of a partially obstructive nature due to displacement or direct invasion by the expanding mass are found in 15% and 10% of patients, respectively.

In those patients in whom tumor necrosis has occurred, a rather constant low-grade fever is common and often accompanied by mild leukocytosis. If tumor erosion into a hollow viscus has occurred, the patient may present with acute or chronic gastrointestinal bleeding. Very rarely patients may manifest hypoglycemia, either due to the production of insulinlike substances by some poorly differentiated sarcomas, or more rapid use of glucose stores by some metabolically active larger sarcomas. Most symptoms of this disease therefore are subtle not pathognomonic and only bring the patient to medical attention late in the course of the disease.[2]

Address reprint requests to F. Kristian Storm, M.D., Department of Surgery, University of Wisconsin, CSC H4/724, 600 Highland Ave., Madison, WI 53792.

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Evaluation of a Retroperitoneal Mass

Abdominal masses generally can be diagnosed by a careful history and physical examination combined with radiographic studies, such as upper gastrointestinal series, barium enema, intravenous pyelogram, and chest x-ray; these studies also will often provide valuable additional information if the diagnosis of sarcoma is made later.

The most definitive single radiographic study is an abdominal computed tomography (CT) scan. The CT will establish the solid composition, the presence or absence of necrosis, evidence of liver metastases, and the retroperitoneal location of such tumors. Because mesenchymal tumors frequently are heterogenous and comprised of irregular solid, semisolid, and liquefactive areas due to patchy necrosis, sarcoma may be highly suspect based on this study alone (Fig. 1).[3] The abdominal CT scan also will be valuable in differentiating sarcomas from other retroperitoneal malignancies, such as lymphoma manifesting multinodal involvement, where lymph node or bone marrow biopsy may be diagnostic, or urogenital tumors that tend to be more homogenous where germ cell markers (*i.e.*, αFP, βHCG) or testicular ultrasound may preclude laparotomy.

If the diagnosis of sarcoma seems likely, or remains possible, an abdominal magnetic resonance image (MRI) scan may better delineate the nature and extent of the neoplasm. Overview coronal sections may show the location and patency of the vena cava (Fig. 2). The T_1-weighted MRI image (Fig. 3) often will better define the relationship of the tumor to other solid organs (*e.g.*, liver, spleen, kidney). The T_2-weighted MRI image will best indicate adjacent muscle invasion and is particularly useful in determining the extent of tumor within the psoas or quadratus muscles and those tumors alarmingly near the spinal foramena (Fig. 4).

If the index of suspicion for sarcoma remains high, an aortogram, with or without venacavogram, depending on the location of the tumor, is often warranted (Fig. 5).[4] Sarcomas tend to be quite vascular neoplasms and knowledge of the extent and location of tumor-feeding vessels are important aids for operative planning. Displacement, encasement, and/or direct invasion of normal vessels may be delineated. The aortogram also will evaluate renal function should the patient require unilateral nephrectomy.

Finally, before biopsy, a chest CT scan should be obtained to rule out pulmonary metastases. Retroperitoneal sarcomas have an equal chance of metastasizing to liver or lung. Not infrequently pulmonary metastases from soft-tissue sarcomas occur only in the lung periphery and are not seen on plain chest x-ray. Chest CT often will disclose peripheral metastases of 1 cm or less (Fig. 6), which could influence future operative management.

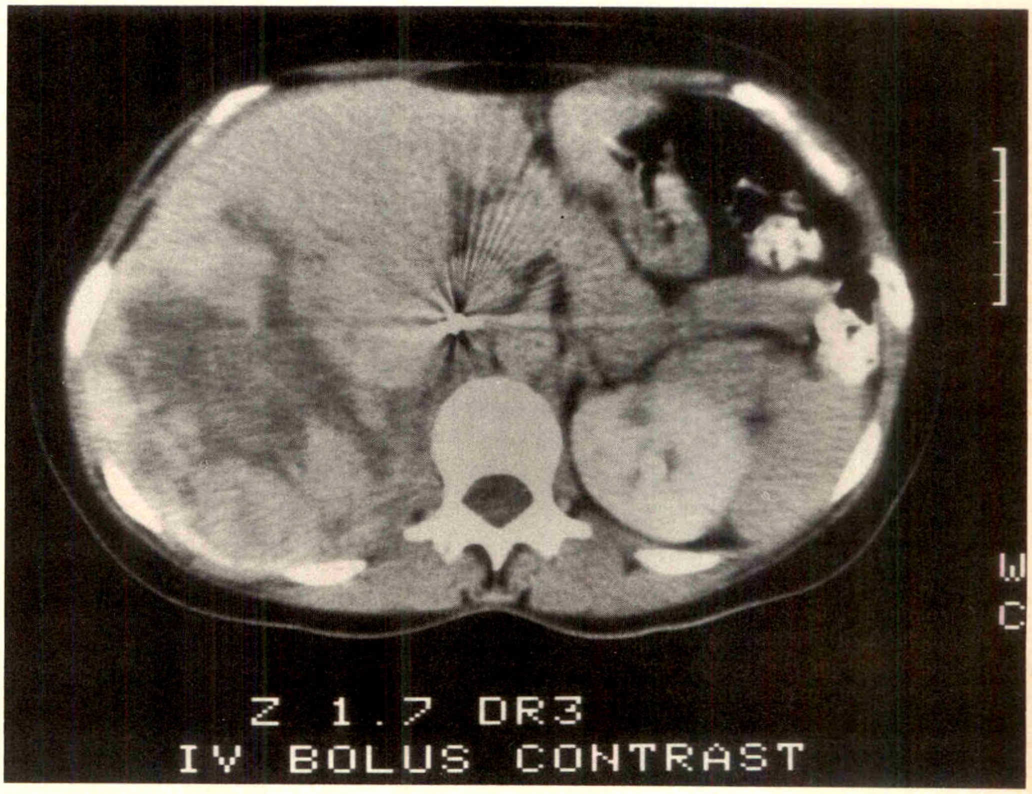

FIG. 1. Abdominal computed tomography scan of retroperitoneal sarcoma indicating its heterogenous nature.

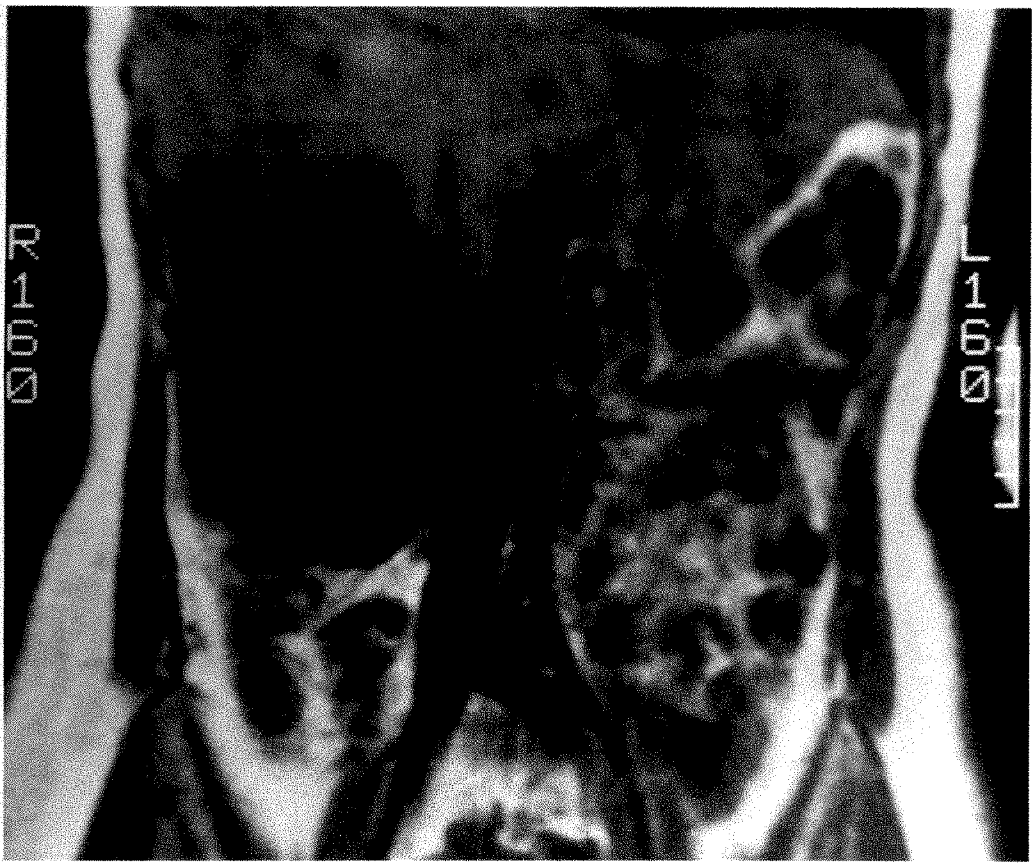

FIG. 2. Magnetic resonance imaging scan, overview coronal section, of retroperitoneal sarcoma shown in Figure 1 indicating patency of the vena cava.

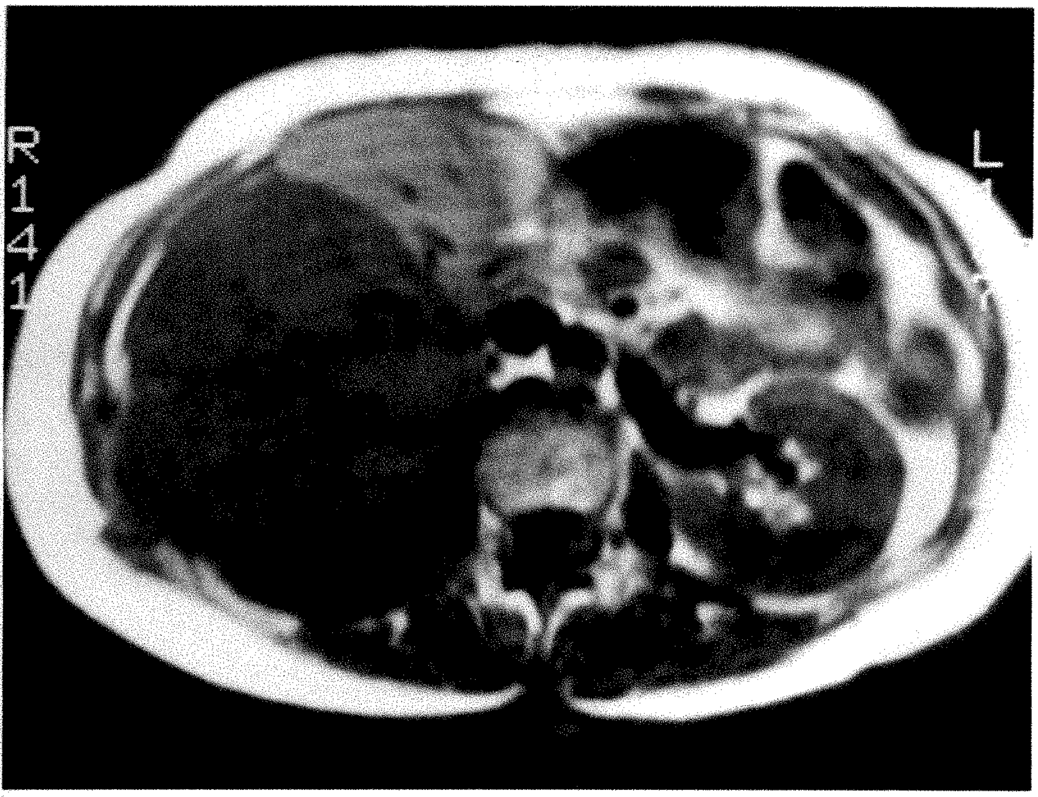

FIG. 3. T1-weighted magnetic resonance imaging scan defining relationship of retroperitoneal sarcoma shown in Figure 1 to adjacent solid organs.

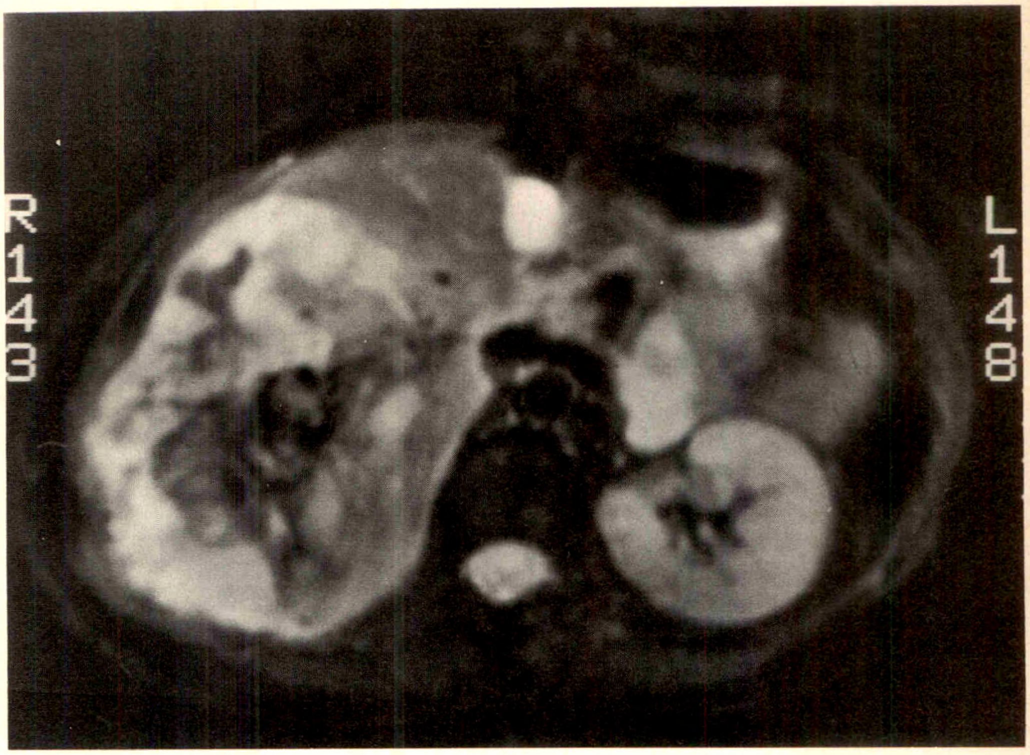

FIG. 4. T2-weighted magnetic resonance imaging scan defining relationship of retroperitoneal sarcoma shown in Figure 1 to adjacent muscle and spinal foramena.

Biopsy of a Retroperitoneal Mass

Ultrasound or CT-guided fine-needle aspiration biopsy or core needle biopsy (*e.g.*, Tru-cut™, Travenol Laboratories, Delfied, IL) often is warranted in confirming deposits of abdominal metastases of other cancers and for diagnosing isolated defects within the liver, adrenal glands, and so on. However these biopsy techniques rarely yield enough useful tissue to provide the information necessary to manage primary retroperitoneal neoplasms successfully, whether they are sarcomas, lymphomas, or urogenital neoplasms. Thus, in the absence of compelling reasons to the contrary, open biopsy is indicated.

In one study of patients with retroperitoneal sarcoma, none manifested intra-abdominal sarcomatosis without having previously undergone biopsy,[2] the implication being that the technique of biopsy is critical in preventing peritoneal seeding of tumor. Laparotomy under general anesthesia is warranted. Wound edges should be protected with plastic barriers. The most solid portion of the tumor should be isolated with a liberal use of laparotomy pads and all normal structures packed off. The tumor capsule should be incised carefully and a 1- to 2-cc wedge of underlaying tumor excised, then the capsule meticulously reapproximated with fine sutures after complete tumor hemostasis has been achieved. Every precaution should be used to prevent free tumor spill or tumor spread *via* an expanding hematoma. The tumor should be sent fresh to the pathologist for 'touch preps' and frozen section on a portion of the sample. In many cases, even though sufficient viable tumor tissue is present for diagnosis, a definitive diagnosis cannot be rendered with certainty by frozen section due to freezing artifact. In this circumstance the operation should be terminated. Treatment should await permanent sections and special stains and electron microscopy as needed. Further dissection or any form of

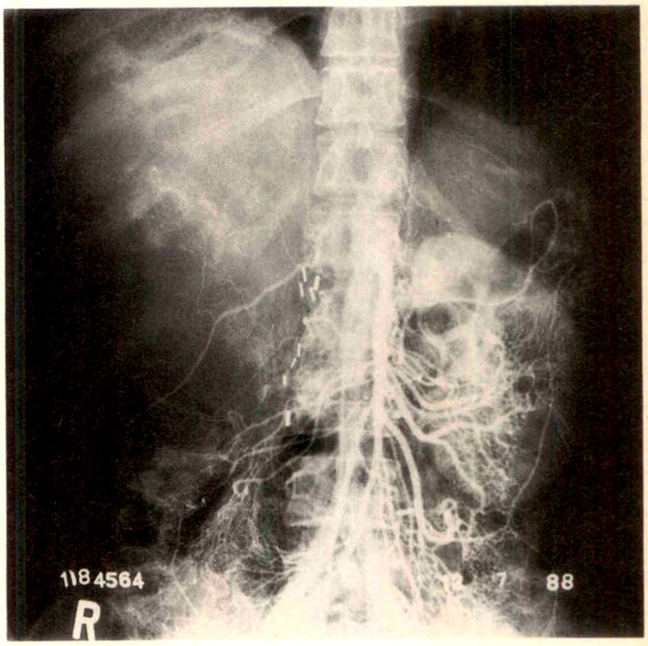

FIG. 5. Aortogram of retroperitoneal sarcoma shown in Figure 1 indicating location and extent of tumor feeding vessels.

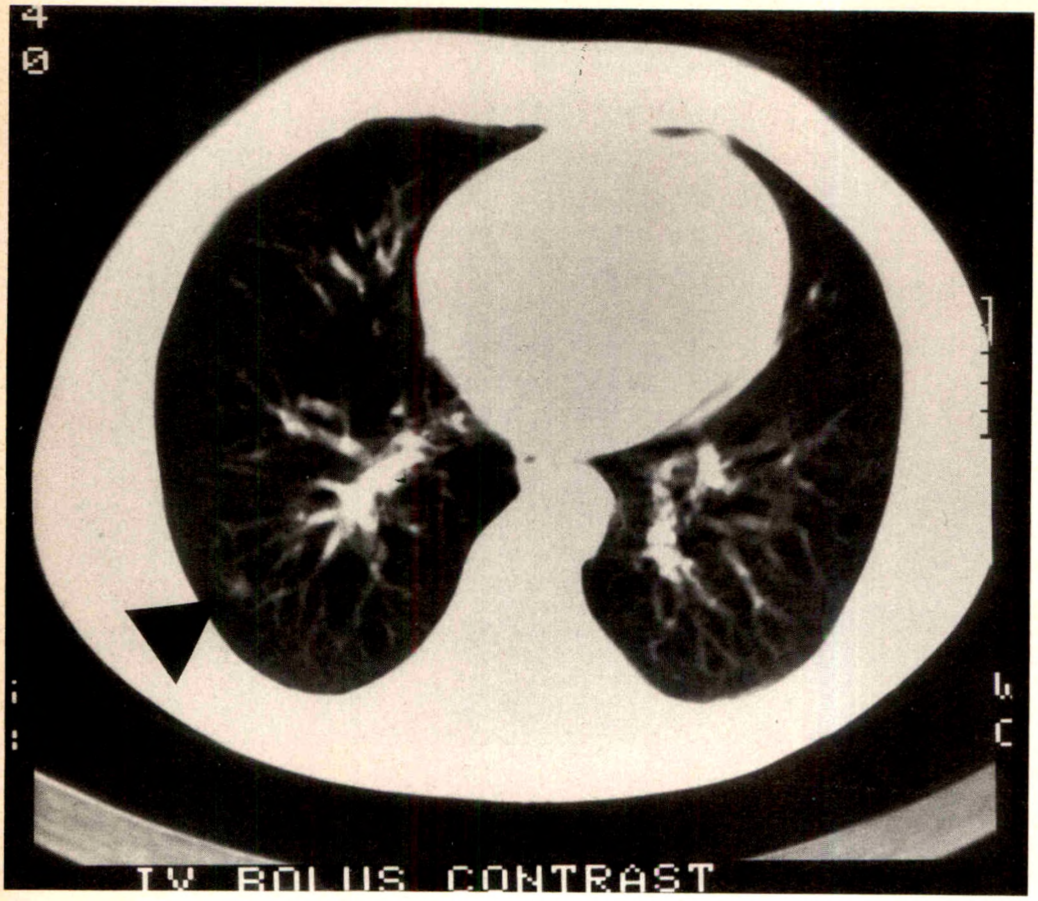

FIG. 6. Chest computed tomograph, lung windows, indicating presence of a 1-cm sarcoma peripheral lung metastasis not seen on plain chest x-ray.

resection is contraindicated in the absence of a definitive diagnosis because such maneuvers inadvertently may expose normal tissue planes to sarcoma, making a subsequent curative resection less likely.

Tumor Histology and Patient Staging

Based on cumulative series (n = 449),[1] the most common retroperitoneal sarcomas are, in descending order of frequency, liposarcoma, fibrosarcoma, leiomyosarcoma, undifferentiated sarcoma, neurosarcoma, and other less common sarcomas (Table 1). Conspicuous by its absence from this list is malignant fibrous histiocytoma, which is a more recent designation being used with greater and greater frequency to identify mesenchymal tumors at all locations but that was not used in most historic series. It is not the variety of sarcoma but rather the histopathologic grade of malignancy, however, that has the greatest bearing on prognosis.

TABLE 1. *Retroperitoneal Sarcoma*

Histology	Incidence (%)*
Liposarcoma	23
Fibrosarcoma	19
Leiomyosarcoma	16
Neurosarcoma	12
Undifferentiated	16
Other, various	14

* Combined series, n = 449.

Sarcomas are designated as grade 1, G1 (well differentiated), grade 2, G2 (intermediate grade, moderately well differentiated), or grade 3, G3 (poorly differentiated or undifferentiated) depending on the degree of cytologic atypia, number of mitoses, and presence and extent of necrosis. Tumors measuring less than 5 cm are designated T1, ≥ 5 cm are T2, and those with bone, nerve, or vascular invasion were classified T3 in older schema.

Staging of patients with soft-tissue sarcoma has been in transition in the past decade. As a general rule, the histopathologic grade of the tumor determines the stage of the patient's disease (Table 2). A patient with a G1 sarcoma is stage I, and so on. Patients with a 'T1' tumor less than 5 cm are substage A; those with larger tumors are substage B. In some past schemas, patients with regionally invasive (T3) tumors were classified as substage IV A; currently stage IV A identifies patients with lymph node metastases and IV B identifies patients with distant metastases.

TABLE 2. *Sarcoma Staging*

Tumor Designation	Tumor Histopathology	Tumor Grade	Patient Stage
G1	Well differentiated	Low	I
G1	Mod. well differentiated	Intermediate	II
G3	Poorly differentiated; undifferentiated	High	III

<5 cm = T1 tumor; substage A.
≥5 cm = T2 tumor; substage B.
(Bone, nerve, vessel invasion = T3 tumor; substage IVA in past schemas).
Currently stage IVA designates patients with nodal metastases; IVB denotes distant disease.

Operative Techniques

Incisions

Long midline or diagonal 'saber slash' incisions are used to provide optimum exposure to retroperitoneal sarcomas, either of which may be extended into the chest as needed. It should be remembered that sarcomas tend to be highly vascular neoplasms whose blood supply almost invariably arises from the midline (Fig. 5). Early control of these midline vessels is mandatory, which is virtually impossible *via* flank approaches. The flank approach is mentioned in this context only to be condemned because it is one of the important contributory factors to poor exposure, inadequate vascular control, unrelenting hemorrhage, and tumor dehiscence.

Evaluation of Resectability

Retroperitoneal sarcomas tend to be either right sided or left sided, with few actually arising from midline structures. Unfortunately the retroperitoneum is not compartmentalized like the extremity, and as a consequence there are no significant fascial barriers to contain the neoplasm. The first maneuver in determining resectability requires dissection, often in a subadventitial plane, along the lateral border of the aorta or vena cava, extended dorsally between the spine and psoas/quadratus muscles (Fig 7). This critical first step will determine whether the tumor has traversed along a spinal nerve root into the spinal foramena and into the cord, the presence of which would render the tumor incompletely resectable. The second step is to incise peritoneum with a sufficiently deep margin of normal body wall to provide a plane of dissection posteriorly to the spinous processes (Fig. 7). If at this point the surgeon can touch his fingers together bimanually *via* the two dissection planes and find an absence of interviewing tumor, the sarcoma is usually resectable.

A solid or hollow organ attached to the neoplasm should be considered potentially invaded and that normal structure, or portion thereof, should be resected *en bloc* with the specimen, which may include kidney, spleen, gallbladder, and portions of the diaphragm, liver, stomach, pancreas, gut, and so on. In general sarcomas do not directly invade major vessels, nerves, or bone. However su-

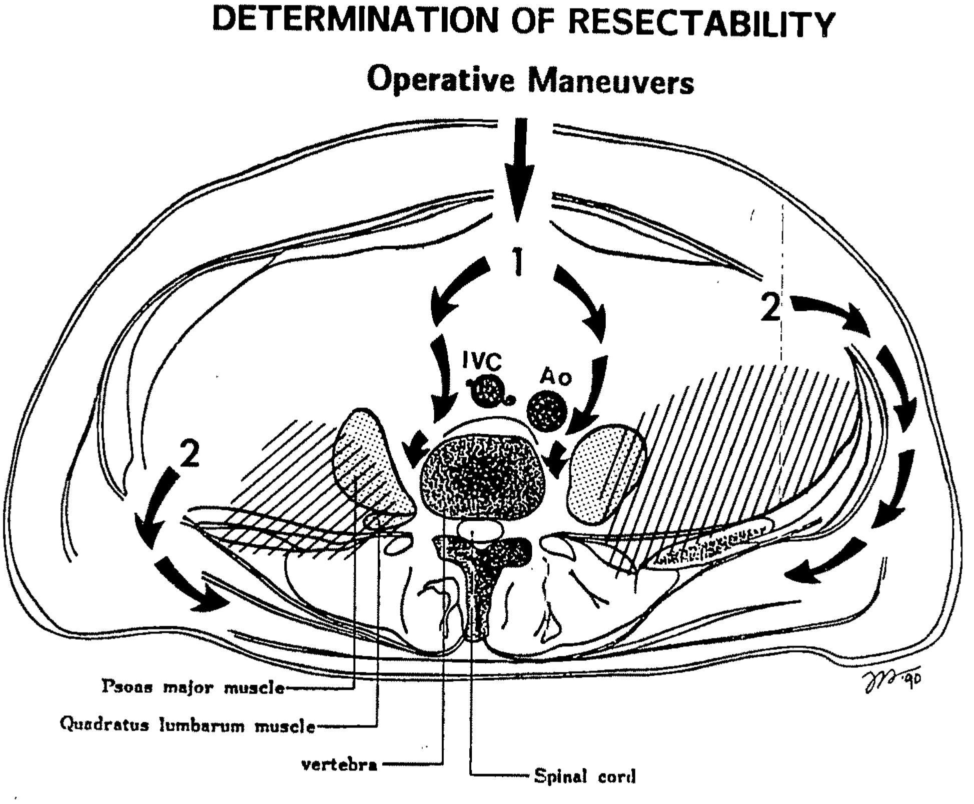

FIG. 7. Initial intraoperative maneuvers to assess resectability, particularly regarding evaluation of areas adjacent to spinal foramena.

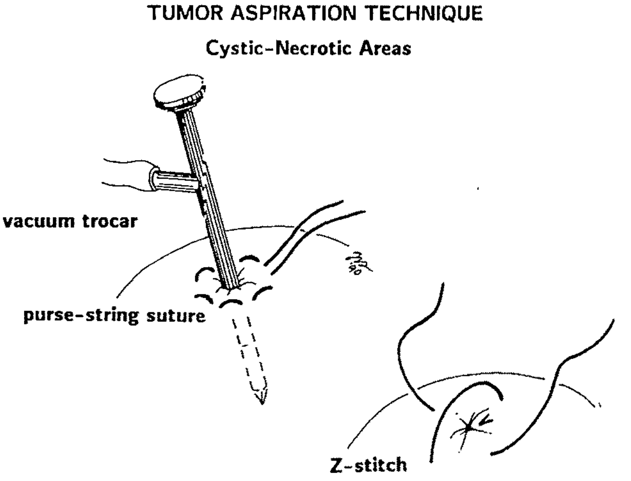

FIG. 8. Decompression of cystic tumor area using aspirating trocar and purse-string suture; an additional Z stitch is used to bury the puncture site.

badventitial, subperineural, and subperiosteal dissections may provide the only margins. Because of the development of extensive venous collaterals from vena caval compression, the vena cava can be resected safely without reconstruction in most cases. Aortic resection and replacement may be required in selected patients with very rare sarcomas arising from or encasing this structure.

Retroperitoneal sarcomas are frequently under extreme pressure and can be quite tense. This fact, when associated with large areas of liquefactive necrosis, makes tumor dehiscence likely during even minimal manipulation. In such cases safe resection often can be facilitated by decompressing large cystic areas within the tumor *via* a vacuum trocar and purse-string suture (Fig. 8). Again great care should be used to avoid tumor spill, and the puncture site should be buried *via* an additional Z stitch.

Results of Therapy

Resectability

Data collected during the past two decades from combined series (n = 560)[2,5–11] indicate that only one half the patients explored for retroperitoneal sarcoma undergo complete tumor resection, defined as removal of all gross disease (Table 3). Some form of partial resection or debulking has been undertaken in about one fifth of patients. These rates of resection have not changed significantly in time.

TABLE 3. *Resectability Rates*

Author (reference)	Study Dates	No. Pts.	Resection Complete	Resection Partial	Biopsy Only
Braasch and Mon[5]	1937–1967	37	15	7	15
Cody et al.[6]	1961–1971	34	13	21*	
	1971–1977	68	45	23*	
Storm et al.[2]	1964–1979	54	33	5	16
Karakousis et al.[7]	1957–1980	68	27	7	34
Dalton et al.[8]	1963–1982	116	63	25	28
McGrath et al.[9]	1964–1982	47	18	18	11
Glenn et al.[10]	1975–1983	50	37	8	5
Jaques et al.[11]	1977–1987	86	43	34	9
Total		560	294 (53%)	104 (19%)	118 (21%)

* Includes both partially and nonresected tumors.

Survival

The mean overall survival rate for patients presenting with retroperitoneal soft-tissue sarcoma is shown in Figure 9. Data from combined series (n = 410)[4,6–9,11] indicate 2-, 5-, and 10-year survival rates of 56%, 34%, and 18%, respectively.

The mean overall survival rate of patients undergoing complete (n = 240) *versus* incomplete (n = 228) resection is shown in Figure 10.[6–9,11,12] Even with complete resection, only slightly more than one half the patients survive 5 years. At each point in time, patients who have had resection of all gross disease have fared significantly better than those not completely resected.

Further analysis of patients who underwent incomplete resections appears to indicate some advantage to partial resection over biopsy alone at 2 years (52% *versus* 25% overall survival), but any benefit seems to be lost by 5 years (Fig. 11).[7,9,11]

The overall survival by histopathologic tumor grade of patients with completely resected disease is shown in Figure 12. Low-grade sarcomas, which tend to be fibrosarcomas and liposarcomas, appear to have an improved prognosis over high-grade (G2, 3) lesions,[2,6,8] as is true of sarcomas arising at other sites.

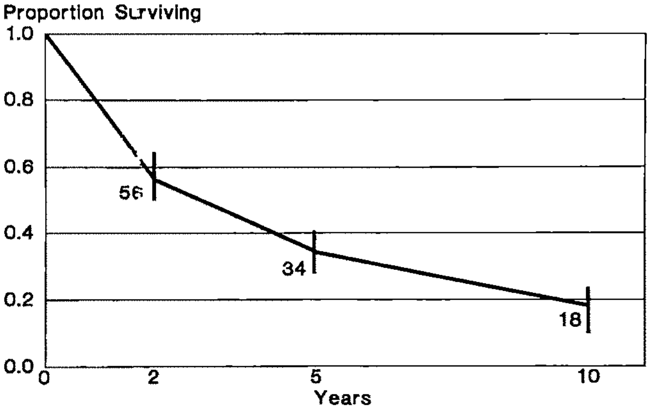

FIG. 9. Overall survival rates in patients presenting with retroperitoneal soft-tissue sarcoma (averages and ranges from cumulative series, n = 410).

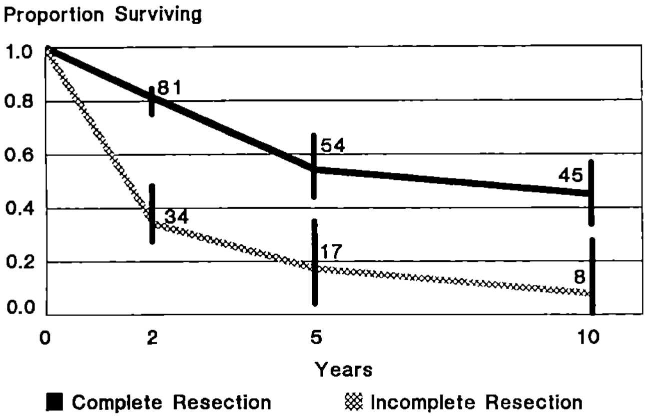

FIG. 10. Overall survival rates by extent of resection. (Averages and ranges from cumulative series. Complete resection, n = 240; incomplete resection, n = 228.)

Recurrence After Complete Resection

Unfortunately more than 90% of patients undergoing successful removal of all gross tumor develop locally recurrent disease by 10 years (Fig. 13).[2,6-9,11] Because on the average only one third of patients ever manifest distant disease (the vast majority in liver or lung), local recurrence is the usual cause of death.[2,8,9,11]

Results of Adjuvant Radiation Therapy

Adjuvant radiation therapy has been used by several centers after complete resection (Table 4). External beam radiation appears to have had no impact on local control or survival,[6,7,9,10] which has been thought to be due to dose-limiting toxicity of the bowel. Unfortunately intraoperative radiotherapy (IORT) combined with external beam therapy to an accumulative dose of 60 Gy has also resulted in a similar pattern of local failure and no improvement in survival to date.[13]

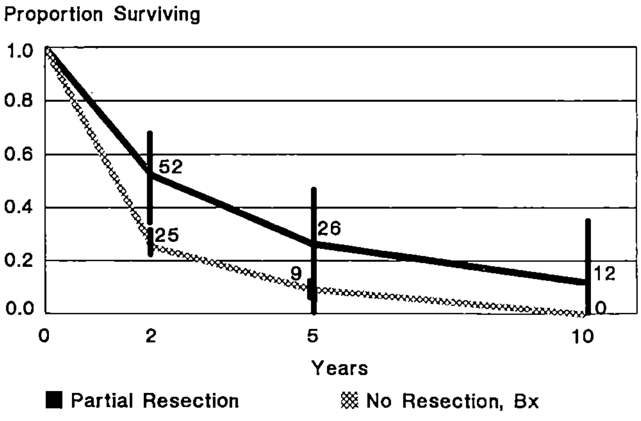

FIG. 11. Overall survival rates by extent of incomplete resection. (Averages and ranges from cumulative series. Partial resection, n = 59; biopsy only, n = 54.)

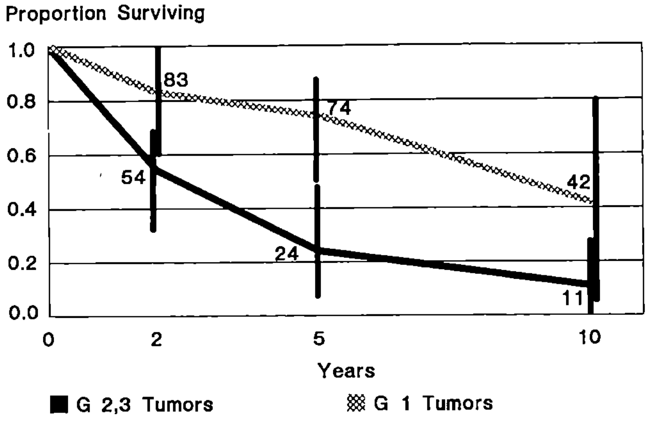

FIG. 12. Overall survival rates by histopathologic tumor grade in completely resected tumors. (Averages and ranges from cumulative series. G1 tumors, n = 49; G2, 3 tumors, n = 80.)

Results of Adjuvant Chemotherapy

Few trials exist using adjuvant chemotherapy combined with complete resection. Doxorubicin-based regimens, historically shown to have significant activity in metastatic sarcoma,[14] have had no impact on survival in the adjuvant[10] or neoadjuvant setting[2] on primary sarcomas arising in the retroperitoneum (Table 5).

Future Priorities

More than 90% of patients with completely resected retroperitoneal sarcomas fail locally. Unfortunately it is unclear from published series whether tumors recur after total resection with negative microscopic margins. It is also unknown what margin is necessary to achieve tumor clearance. The role of partial resection in these highly vascular and frequently necrotic tumors has also yet to be defined. For these as well as a number of other obvious reasons, a national retroperitoneal sarcoma registry needs

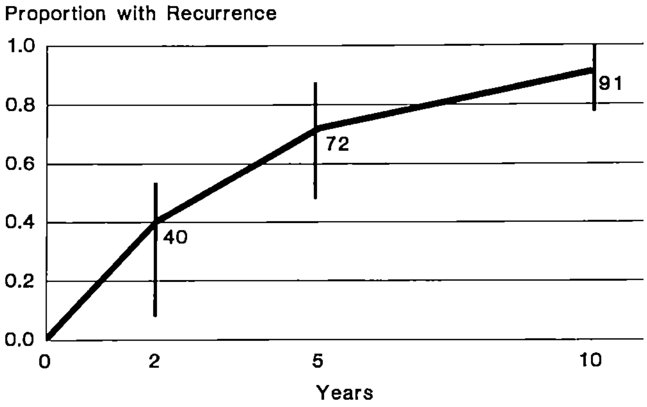

FIG. 13. Incidence of local recurrence after complete resection (averages and ranges from cumulative series, n = 204).

TABLE 4. *Postoperative Adjuvant Radiotherapy After Complete Surgical Resection*

			Overall Survival			
Author (reference)	Study Dates	No. Pts.	Surgery Alone (%)	RT ± Chemo (%)	Intraop RT (%)	p Value
Cody et al.[6]	1951–1974	38	30	53	—	NS @ 5 yrs.
Karakousis et al.[7]	1957–1980	27	75	75	—	NS @ 5 yrs.
McGrath et al.[9]	1964–1982	18	75	50	—	NS @ 5 yrs.
Glenn et al.[10]	1975–1980	37	43*	43	—	NS @ 3 yrs.
Kinsella et al.[13]	1980–1985	35	43*	48	48	NS @ 3 yrs.

* Survival at 3 years from cumulative surgical series, n = 80, high-grade tumors.[2,6,8]

NS, not significant; RT, radiation therapy; Chemo, chemotherapy.

TABLE 5. *Adjuvant Chemotherapy with Complete Surgical Resection*

			Overall Survival			
Author (reference)	Study Dates	No. Pts.	Surgery Alone (%)	RT Alone (%)	Chemo (± RT) (%)	p Value
Glenn et al.[10]	1975–1980	37	54*	60	60	NS @ 2 yrs.
Storm et al.[2]	1976–1981	10	54*	—	60	NS @ 2 yrs.

* Survival at 2 years from cumulative surgical series, n = 80, high-grade tumors.[2,6,8]

NS, not significant; RT, radiation therapy; chemo, chemotherapy.

to be established to evaluate and track these unusual neoplasms.

A better understanding of appropriate biopsy techniques and intraoperative approaches on the part of the surgeon will clearly enhance resectability and therefore prolong survival; however the few recorded attempts to improve local disease control using adjuvant radiation therapy or chemotherapy have largely failed. It is certain that the bowel will not tolerate high-dose external beam radiation therapy and that IORT may obviate this toxicity. Regrettably no pilot studies or prospective randomized trials have existed to explore adjuvant or neoadjuvant combined radiotherapy plus chemotherapy, which has been found to be so useful in achieving local control (88 of 96 patients, 92%) and limb salvage (95 of 96 patients, 99%) in intermediate and high-grade extremity soft-tissue sarcomas.[15] Despite continuing advances in radiation delivery and the development of new chemotherapeutic agents that require testing, these rare tumors do not lend themselves well to individual institutional trials. It is apparent that collective efforts will be necessary to alter the course of this disease. Experts in the field need to establish a national intergroup study to develop strategies and innovative trials for effective treatment of these lethal tumors arising in the retroperitoneum.

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ORIGINAL ARTICLES

Verification of a New Clinicopathologic Staging System for Colorectal Adenocarcinoma

FABRIZIO MICHELASSI, M.D., F.A.C.S.,[*] JAIME J. AYALA, M.D.,[*] TOMMASO BALESTRACCI, M.D.,[*]
RUTH GOLDBERG, M.D.,[†] RICHARD CHAPPELL,[‡] and GEORGE E. BLOCK, M.D., F.A.C.S.[*]

From the Departments of Surgery,[*] Pathology,[†] and Statistics,[‡] University of Chicago, Chicago, Illinois

Rectal adenocarcinoma is said to have a poorer outcome than colon adenocarcinoma when compared on the basis of Dukes' staging. However a new staging system, determined by a multivariate analysis of 147 patients with rectal adenocarcinoma, has revealed three other variables significantly related to outcome. Therefore this study analyzed the authors' experience with colonic carcinoma during the same time period as they had studied for rectal carcinoma to determine whether the new staging system is valid for colon carcinoma as well, and, if so, to compare the outcome of patients with colon and rectal carcinoma on the basis of this new staging. A total of 603 patients with 611 colonic adenocarcinoma were operated on at the University of Chicago Medical Center between 1965 and 1981. Two hundred seventy-nine adenocarcinomas (45.7%) were located proximal to the splenic flexure and 332 (54.3%) were located between the splenic flexure and the rectosigmoid. Four hundred sixty-two patients underwent segmental colectomy, 46 subtotal colectomy, 26 total colectomy, 18 proctocolectomy, 5 abdominal-perineal resection, 1 appendectomy, while 20 had local excision of the tumor through colotomy and 25 had permanent diverting stoma as the only procedure. The operative mortality rate was 6.1% in the whole group, but was only 2.7% in the group of potentially curable patients. Complete follow-up was obtained in all patients. To validate a previous staging system for Dukes' B and C rectal adenocarcinoma, the authors investigated the correlation between 5-year survival for colonic carcinoma patients and all relevant variables that they had considered potentially meaningful in the previous study with rectal adenocarcinoma. The resulting multivariate analysis using Cox regression showed that the four variables found previously to be significantly related to outcome for rectal adenocarcinoma patients (stage, race, tumor morphology, and vascular and/or lymphatic microinvasion) were the only four variables significantly ($p < 0.05$) associated with outcome for colonic adenocarcinoma patients. In addition, by using the results of their previous staging system for rectal adenocarcinoma patients, they 'predicted' the 5-year survival rates of the colon adenocarcinoma patients, divided in 16 staging subgroups. In subgroups of at least 15 patients, the rectal staging system predicted the outcome to within 1 to 6 percentage points of the observed outcome of the colonic adenocarcinoma patients. Thus this study validates this staging system, incorporating stage, race, tumor morphology, and microinvasion to predict 5-year survival rate more accurately than Dukes' staging alone for both colon and rectal adenocarcinoma. In addition, when grouped according to this classification, patients with colon carcinoma have the same outcome probabilities as patients with rectal carcinoma.

THE ORIGINAL DUKES' classification has been modified and refined several times[1] in the 50 years following its publication. Although the modified classifications have permitted a more precise prognosis in patients with 'early' cancers confined to the mucosa (stage A), and in patients with more extensive and distant spread (stage D), the remaining patients, who represent the majority of colorectal cancers, are grouped into two large, heterogeneous groups.

In 1988, prompted by our inability to predict accurately the long-term prognosis of an individual patient after resection of a stage B or C rectal carcinoma, we evaluated our experience with rectal carcinoma to identify clinical or pathologic characteristics that influenced outcome.[2] The results of our study on rectal carcinoma revealed that Dukes' staging, race, tumor morphology, and the presence or absence of lymphatic and/or vascular microinvasion significantly influenced patient outcome. By associating these four statistically significant and independent variables, we were able to divide patients with stage B and C rectal adenocarcinoma into 16 subgroups, each with its own more precise prognosis. The present study represents our attempts to validate these results, to extend them to colon carcinoma, and to compare the long-term prognosis

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Address reprint requests to Fabrizio Michelassi, M.D., The University of Chicago, Department of Surgery, 5841 South Maryland Ave., Chicago, IL 60637.

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of patients with colon and rectal carcinoma on the basis of this new staging.

Materials and Methods

Between 1965 and 1981, 603 patients with colonic adenocarcinomas underwent surgical treatment at the University of Chicago Medical Center. The clinical records of all these patients were reviewed and complete follow-up to December 1988 was obtained in all through the Registry of Neoplastic Diseases of the University of Chicago. Data on age, sex, race, tumor location, morphology and size, evidence of local invasion, type of operation, mortality, evidence of local recurrence or distant metastasis, presence of synchronous colonic adenocarcinomas, familial polyposis, and ulcerative or Crohn's colitis were specifically sought in each instance. Tumors were defined as exophytic when they exhibited a polypoid growth pattern protruding into the lumen at least 1 cm; when lacking such characteristic growth pattern, tumors were classified as nonexophytic. Operative deaths were defined as those deaths occurring within 30 days from the time of operation.

Histologic slides and archival paraffin blocks were retrieved for confirmation of diagnosis, determination of histologic type, stage, degree of tumor differentiation, and presence of vascular and/or lymphatic microinvasion by one pathologist (R. Goldberg) who was unaware of the patients's clinical course. Lymphatic microinvasion was defined as the presence of tumor within an endothelial lined space lacking a smooth muscle coat; the same finding was defined as vascular microinvasion if the endothelial line space was surrounded by a smooth muscle layer. Tumors were staged according to the Astler-Coller modification of Dukes' classification.[3]

Data regarding the occurrence of distant metastases, the incidence of local recurrence, and presence of tumor extension through the bowel wall were analyzed by the Kruskal-Wallis analysis of variance (ANOVA),[4] considering each independent variable individually.

To validate the clinical pathologic staging described for potentially curable rectal adenocarcinoma by us in 1988[2] and extend it to colonic adenocarcinoma, we analyzed the 5-year survival of the patients with colonic adenocarcinoma as we did for those with rectal adenocarcinoma. All references to patients with rectal adenocarcinoma refer to our previously described cohort of 250 patients with rectal adenocarcinoma operated on at the University of Chicago Medical Center from 1965 to 1981.[2]

A logistic regression analysis was used, with the dependent variable being 5-year survival. Our previously reported techniques[2] for estimation of parameters and confirmation of assumptions were repeated for this analysis. The same independent variables considered in our previous analysis with rectal adenocarcinoma patients were entered into the multivariate analysis: age (both as a continuous variable and divided into three groups (less than 40 years, between 40 and 59 years, and more than 60 years)*, sex, race, Dukes' stage, tumor morphology (exophytic *versus* nonexophytic), mucin production, and the presence or absence of vascular and/or lymphatic microinvasion. In addition we also considered location of the tumor along the colon (cecum, ascending with hepatic flexure, transverse with splenic flexure, descending, sigmoid, and rectosigmoid).

In addition, to determine whether patients with colon cancer had similar 5-year survival probabilities to patients with rectal cancer when all four variables significant for survival were considered, we 'predicted' the outcome of the colonic carcinoma patients based on the results of the previous model using rectal adenocarcinoma patients alone for each of the 16 subgroups and compared the results to the corresponding observed outcomes of colonic carcinoma patients.

Results

Clinical Findings

Of the 603 patients, 291 were men and 312 were women. Mean age at the time of operation was 64.4 years, with a range from 15 to 93 years. The 603 patients considered had a total of 611 adenocarcinomas. Of these, 279 (45.7%) were located proximal to the splenic flexure and 332 (54.3%) between the splenic flexure and the rectosigmoid (Fig. 1). Twenty patients had associated ulcerative colitis, three patients Crohn's colitis, and four familial polyposis. Thirty-three patients presented with a total obstruction and 14 with an open perforation.

The location of the tumor within the colon and presence of synchronous tumors or associated conditions influenced the type of operation that was performed. One patient underwent an appendectomy, 462 patients a segmental colectomy, 46 a subtotal colectomy, 26 a total colectomy, 18 a protocolectomy, 5 an abdominal perineal resection, while 20 had a local excision of the tumor through a colotomy and 25 a permanent diverting stoma as the only procedure.

Follow-up analysis revealed that 37 patents (6.1%) died in the immediate postoperative period. Of these only one third (n = 13) had a potentially curable adenocarcinoma, for a mortality rate of 2.7% in this group of patients. Furthermore the operative mortality rate decreased markedly during the period reviewed (Fig. 2).

Table 1 compares the distribution, by tumor stage, of

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* Note that in the previous publication, age was entered as a continuous variable and dichotomized at 40 years.

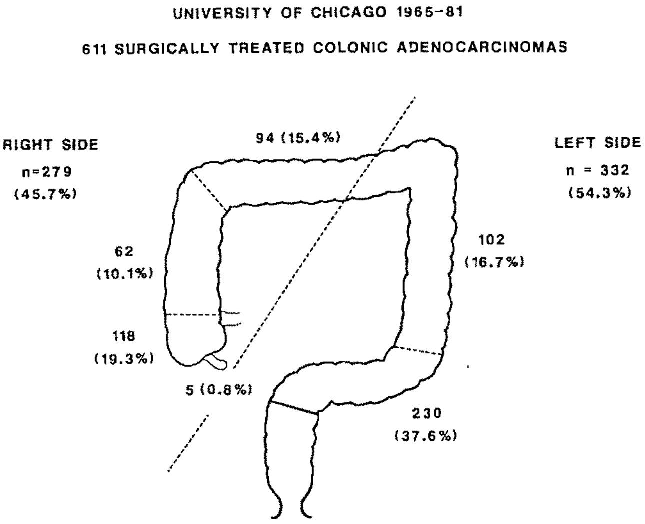


FIG. 1. Location of 611 colonic adenocarcinomas in 603 patients surgically treated at the University of Chicago between 1965 and 1981.

TABLE 1. *Tumor Stage for Colonic and Rectal Adenocarcinoma*

Dukes' Stage	Colon (n = 611)		Rectum* (n = 250)	
A	52	8.5%	25	10%
B_1	37	6.1%	32	12.8%
B_2	218	35.7%	55	20.8%
C_1	—	—	5	2.0%
C_2	173	28.3%	62	24.0%
D	131	21.4%	71	28.4%

* Patients described in detail in previous publication.[2]

colon adenocarcinomas to that of our previously described rectal adenocarcinoma patients. The distributions among classes A, B, C, and D were comparable between groups. However, within stage B, there was a smaller percentage of B_1 tumors and a higher percentage of B_2 tumors among patients with colon cancer compared to those with rectal cancer.

Ten patients had another synchronous tumor, which was an adenocarcinoma in eight cases and a carcinoid in two. Synchronous adenocarcinomas were found, therefore, in 1.3% of patients. There were six cases of synchronous Dukes' B_1 and B_2 adenocarcinomas, one case of synchronous B_2 and C_2, and one case of synchronous C_1 and C_2. Patients with synchronous adenocarcinomas were considered only once in our analysis and were grouped under the higher stage lesion.

Five- and ten-year actuarial survival rates classified by the modified Dukes' staging and exclusive of perioperative deaths are summarized in Table 2 for the colonic adenocarcinoma patients, as well as for our rectal adenocarcinoma patients. Overall patients with colon cancer, when stratified by stage, had higher percentages of 5- and 10-year survival compared to comparably stratified patients with rectal cancer. The differences were most marked when comparing outcomes for stages B_1 and B_2. For example colon adenocarcinoma patients classified as stage B_1 had 100% 5-year survival compared to only 69% for stage B_1 rectal cancer. Figure 3 illustrates the survival curves, based on tumor stage, following the resection of potentially curable colonic adenocarcinomas.

The local recurrence rate after resection of a Dukes' B or C adenocarcinoma was lower among colon carcinoma patients, 7.5% (31 patients), compared to 12% among rectal carcinoma patients. This is also consistent with the finding for both colon and rectal cancers that the more distal the original tumor arose, the higher was the incidence of local recurrence. For tumors of the cecum, the

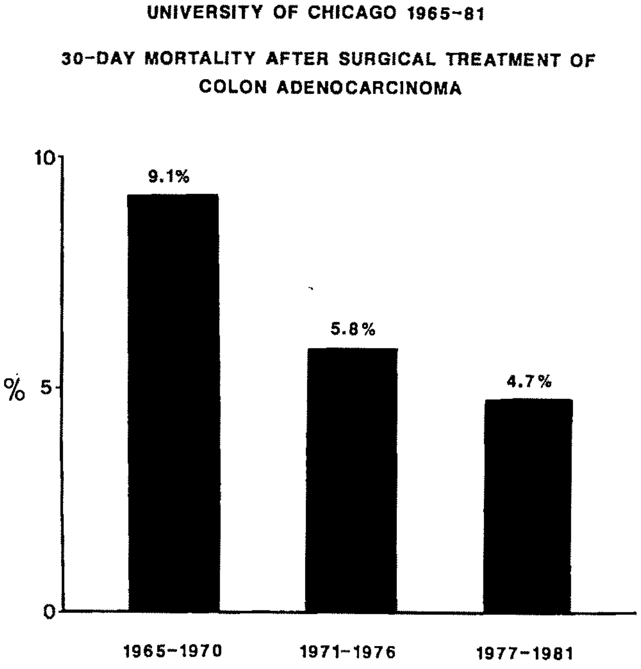


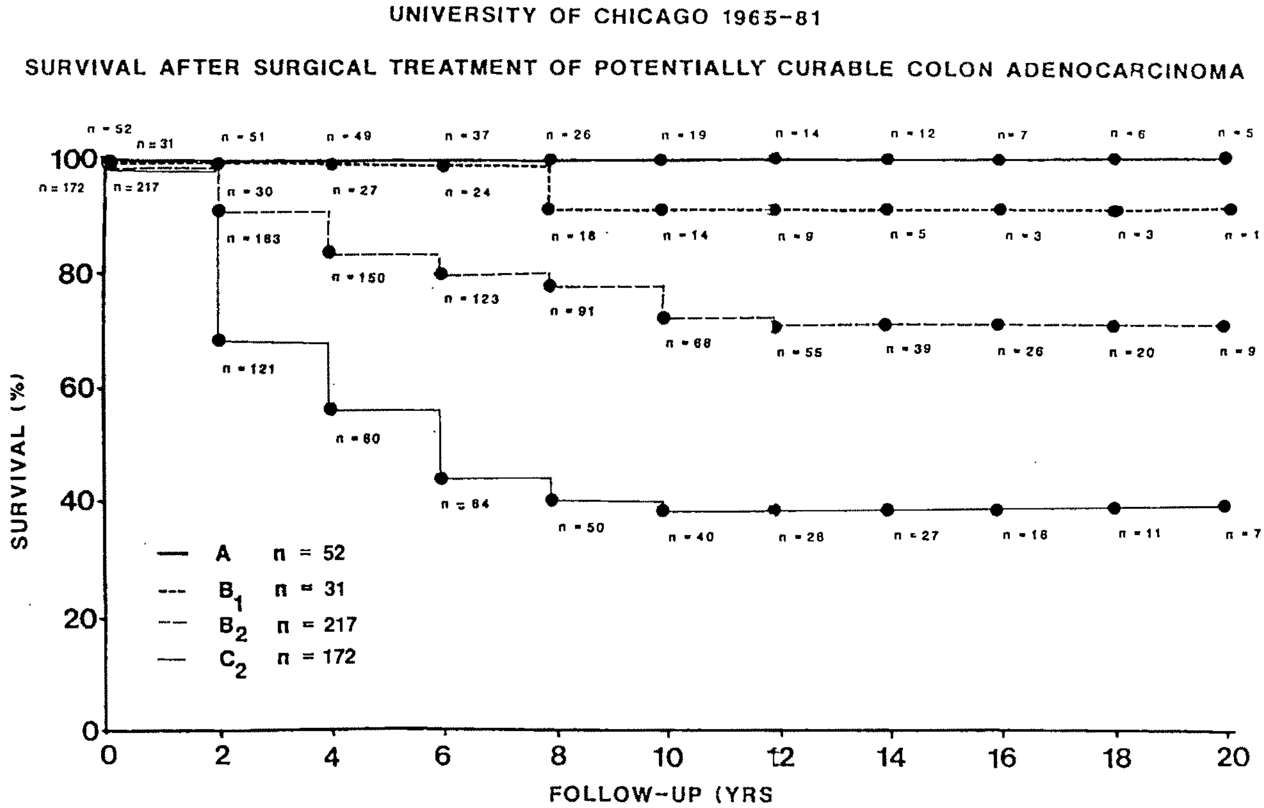
FIG. 2. Operative mortality rate in 603 patients with colon adenocarcinoma surgically treated at the University of Chicago between 1965 and 1981.

TABLE 2. *Five- and 10-year Actuarial Survival Rates*

Dukes' Stage	Colonic Adenocarcinoma* 5 Years (%)	Colonic Adenocarcinoma* 10 Years (%)	Rectal Adenocarcinoma 5 Years (%)	Rectal Adenocarcinoma 10 Years (%)
A	100	100	100	†
B_1	100	95.3	68.8	59.4
B_2	82.7	72.4	55.8	44.2
C	49.1	39.5	42.9	25.4
D	3.7	1.5	2.8	†

* Patients with synchronous lesions are grouped with the higher grade lesion (see text).

† Ten-year actuarial survival rate not reported in previous publication.[2]


UNIVERSITY OF CHICAGO 1965-81

SURVIVAL AFTER SURGICAL TREATMENT OF POTENTIALLY CURABLE COLON ADENOCARCINOMA

FIG. 3. Survival rates by Dukes' stage based on life table analysis calculated for patients after surgical treatment of potentially curable colon adenocarcinoma at the University of Chicago between 1965 and 1981. Patients who died of intercurrent disease were censored at the time of the last known follow-up.

incidence was 4.8%, compared to 7.3% for tumors of the ascending colon, 8.6% for tumors of the transverse and descending colon, and 9.4% for tumors of the sigmoid and rectosigmoid. The local recurrence rate for colon adenocarcinomas was 1.9% for Dukes' A, 6.3% for Dukes' B, and 9.8% for Dukes' C tumors. One half the recurrences were clinically evident by 2 years and 8 months, and 50% of patients died by 3.5 years. The survival rates, based on life table analysis, were 40.5% at 5 years and 21.6% at 10 years.

Distant metastasis developed in 149 patients (32.5%). Their 5-year survival rate, according to the stage of original tumor, was 100% for Dukes' A and B_1 adenocarcinomas, 30.6% for Dukes' B_2, and 15.3% for Dukes' C_2 tumors. In the 81 cases in which the involved site was known, the liver was the single organ involved in 43 cases (29% of patients with metastasis and 7.1% of all patients). The likelihood of developing a distant metastasis was found to be significantly proportional to the stage of the original tumor, 3.6% at stage A and B_1, 22.7% at stage B_2, and 57.6% at stage C_2 ($p < 0.001$, ANOVA). Vascular and/or lymphatic microinvasion was significantly associated with the development of metastasis; 54.8% of patients with this characteristic developed distant metastasis, as compared with 24.9% of patients without microinvasion ($p < 0.01$, ANOVA). Tumors with a nonexophytic growth pattern also developed distant metastasis significantly more frequently than did exophytic tumors (47.8% *versus* 25.9%, respectively; $p < 0.01$, ANOVA).

There was evidence of extension through the bowel wall in 391 patients (82.8%) of the 472 with colonic adenocarcinomas without intraoperative evidence of distant metastasis. Our analysis revealed that the presence of vascular and/or lymphatic microinvasion was associated with a significantly greater incidence of such tumor extension: 95.6% of patients with microinvasion exhibited such evidence, compared with 77.7% of patients without microinvasion ($p < 0.05$, ANOVA). Furthermore a significantly larger percentage of patients with nonexophytic tumors experienced invasion through the bowel wall, compared with those with exophytic tumors (94.9% *versus* 76.6%; $p < 0.05$, ANOVA).

Model Validation

A multivariate logistic regression analysis was performed using the data from the colon adenocarcinoma patients on whom a potentially curative procedure was

TABLE 3. *Factors Influencing 5-year Survival Rate After 'Curative' Resection of Colon Adenocarcinoma According to the Logistic Regression Analysis*

Variable	Beta Coefficient	Standard Error	p Value
Stage			
B (n = 197)	+1.42	0.26	<0.00001
C (n = 151)			
Race			
White (n = 190)	+0.49	0.26	<0.05
Black (n = 158)			
Tumor morphology			
Exophytic (n = 231)	+0.65	0.26	<0.02
Nonexophytic (n = 117)			
Vascular–lymphatic microinvasion			
Absent (n = 252)	+0.92	0.28	<0.001
Present (n = 96)			

performed. Eight patients with synchronous colonic adenocarcinomas and 64 patients with missing data were excluded from this analysis. The patient outcome variable was the 5-year survival rate, while the independent variables were the relevant variables entered into the previous model for rectal carcinoma.[2] Table 3 summarizes the results of the analysis on colonic adenocarcinoma, listing those variables found to be significantly associated with 5-year survival (*i.e.*, those variables with statistically significant coefficients in the model). The results of the multivariate analysis produced the same four variables found previously to be related significantly to survival for rectal adenocarcinoma patients: Dukes' stage (B compared to C), race (white compared to black), tumor morphology (exophytic compared to nonexophytic), and the absence or the presence of vascular and/or lymphatic microinvasion. By categorizing patients by the four characteristics, 16 subgroups emerge with varying prognoses (Fig. 4). Among these groups, white patients with Dukes' stage B, exophytic adenocarcinoma without microinvasion have the best prognosis, while black patients with stage C, nonexophytic tumors with microinvasion have the worst prognosis.

Because our results demonstrated that, classified by Dukes' stage alone, colon cancer patients have better survival rates than rectal carcinoma patients, we wanted to determine if this difference would be maintained using our more precise staging. To do so, we 'predicted' the 5-year survival rates of each of the 16 subgroups of colon adenocarcinoma patients based on the results of the staging system for rectal carcinoma patients and compared them to the corresponding observed rates for the colon patients. Table 4 displays the results. In subgroups of at least 15 patients, the rectal staging system predicted the

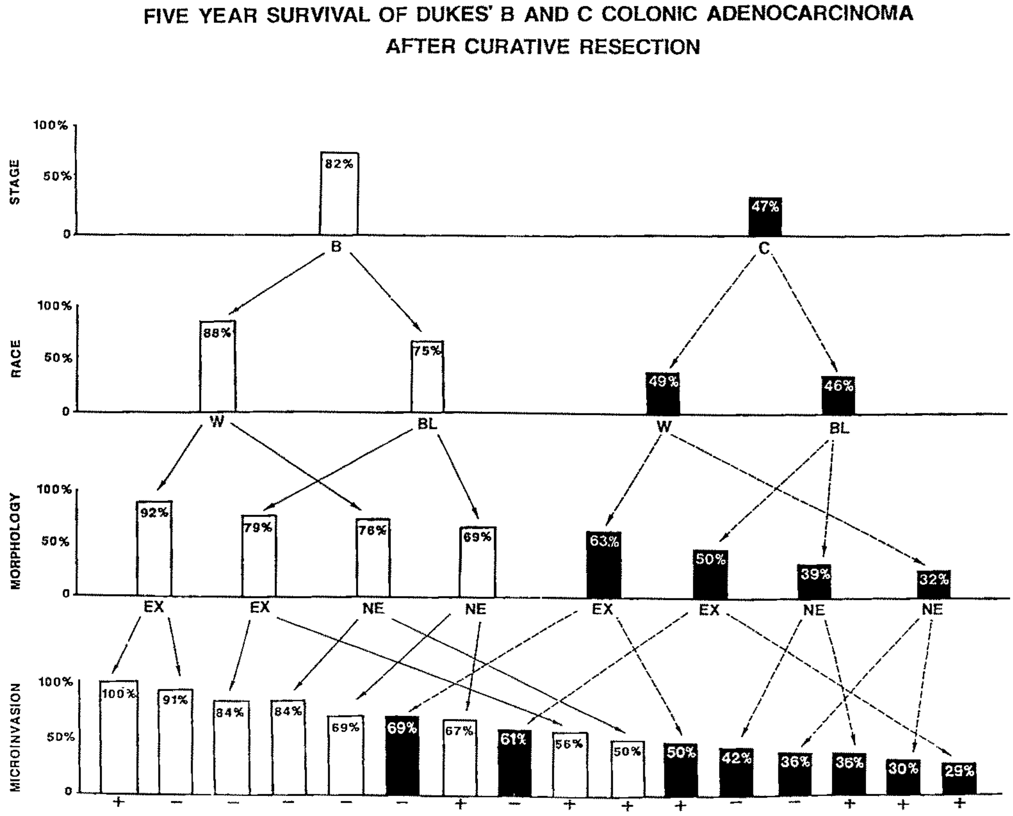


FIG. 4. Refinement of prognostic value of Dukes' classification in estimating 5-year survival probability. Estimates of 5-year survival probabilities, obtained from logistic regression, are shown for 16 different groups of patients. The groups were divided on the basis of those factors (stage, microinvasion, tumor type, and race) believed to have a significant effect on mortality rates. Survival estimates of patients for which all these attributes are known are given at the bottom of the figure. The estimates in the higher levels, for which one or more attributes are not known, were calculated by weighing the estimated probabilities in the constituent groups by those group frequencies in the sample analyzed. Thus these estimates are specific to samples approximately the same composition as the sample considered here. Only the survival estimates given at the bottom can be generalized to accommodate population with other compositions.

TABLE 4. *Predicted and Observed 5-year Survival Rates of 16 Subgroups Based on Staging System, Using Rectal Adenocarcinoma Data to Predict Survival and Colon Adenocarcinoma Data to Observe It*

Stage	Race	Morphology	Micro-invasion	n	Predicted 5-year Survival	Observed 5-year Survival
B	White	Exophytic	−	78	92%	91%
B	White	Exophytic	+	43	85%	84%
B	White	Nonexophytic	−	10	81%	100%
B	White	Nonexophytic	+	19	80%	84%
B	Black	Exophytic	−	29	75%	69%
B	Black	Exophytic	+	9	66%	56%
B	Black	Non-exophytic	−	26	66%	69%
B	Black	Non-exophytic	+	6	59%	50%
C	White	Exophytic	−	31	59%	61%
C	White	Exophytic	+	14	51%	50%
C	White	Nonexophytic	−	14	52%	36%
C	White	Nonexophytic	+	6	40%	67%
C	Black	Exophytic	−	17	33%	29%
C	Black	Exophytic	+	12	32%	42%
C	Black	Nonexophytic	−	20	26%	30%
C	Black	Nonexophytic	+	14	14%	36%

outcome to within 1 to 6 percentage points of the observed outcome of the colonic cancer patients. The greatest difference was noted for the subgroup with the worst prognosis (blacks with stage C, nonexophytic adenocarcinoma with microinvasion). For that group, the predicted value for the 5-year survival was 14% compared to an observed value of 36%. This result suggests that for patients with the worst characteristics, rectal carcinoma may have a worse prognosis than colon carcinoma.

Because our goal has been to develop a more precise predictive staging system, and because both groups demonstrated similar results, we combined the two groups to generate a staging system based on 479 patients for whom sufficient information was available. The results of that regression analysis are presented in Table 5. Figure 5 summarizes the estimates of the 5-year survival probabilities for Dukes' stages B and C colorectal adenocarcinoma, based on the four coefficients obtained from the statistical analysis, for each of the 16 subgroups of patients. In addition we averaged the respective 5-year survival rates of colon and rectal patients to provide probability estimates for Dukes' A and D patients as well. Apart from stages A and D, the estimates range from a high of 91.3% 5-year survival for white patients with no microinvasion, exophytic tumor, stage B to a low of 19.1% for black patients with microinvasion, nonexophytic tumor and stage C.

Of particular note are those subgroups of patients with stage C disease who have higher survival probabilities than other subgroups of patients with stage B. For example both black and white stage C patients without microinvasion and with exophytic tumors have greater survival probabilities than those with stage B disease who have microinvasion and nonexophytic tumors.

Discussion

The present study represents our attempt to validate the results obtained in our initial study on rectal carcinoma. We chose to evaluate patients with colon carcinoma who underwent surgery during the same time period and by the same surgeons at the University of Chicago Medical Center. This group of patients provided us with the opportunity to explore whether our staging, like the Dukes' classification, was valid for colon adenocarcinoma as well as rectal carcinoma, and if so, whether the two sites varied in their prognosis, when controlled for the four significant variables.

The resulting multivariate analysis revealed that the four variables found previously to be significantly related to outcome for rectal adenocarcinoma patients were the only four variables significantly associated with outcome for colonic adenocarcinoma patients. These results confirmed the validity of our staging system and extended its applicability to colon carcinoma.

In addition, in an effort to determine if the site (colon *versus* rectum) influenced outcome, we 'predicted' the outcome for the colon patients based on the rectal model. Presumably, if rectal carcinoma had a uniformly worse prognosis than colon carcinoma, the rectal model would have predicted lower survival rates than that actually observed with the colon patients. In contrast to the results based on Dukes' staging alone, in which we found that rectal patients with stage B or C had worse 5-year outcomes than colon carcinoma patients with the corre-

TABLE 5. *Factors Influencing 5-year Survival Rates After 'Curative' Resection of Colorectal Adenocarcinoma According to the Logistic Regression Analysis*

Variable	Beta Coefficient	Standard Error	p Value
Stage			
B (n = 264)	+1.38	0.22	<0.00001
C (n = 215)			
Race			
Caucasian (n = 277)	+0.6	0.22	<0.007
Black (n = 202)			
Tumor morphology			
Exophytic (n = 289)	+0.79	0.22	<0.0004
Nonexophytic (n = 190)			
Vascular–lymphatic microinvasion			
Absent (n = 295)	+1.02	0.23	<0.00001
Present (n = 184)			

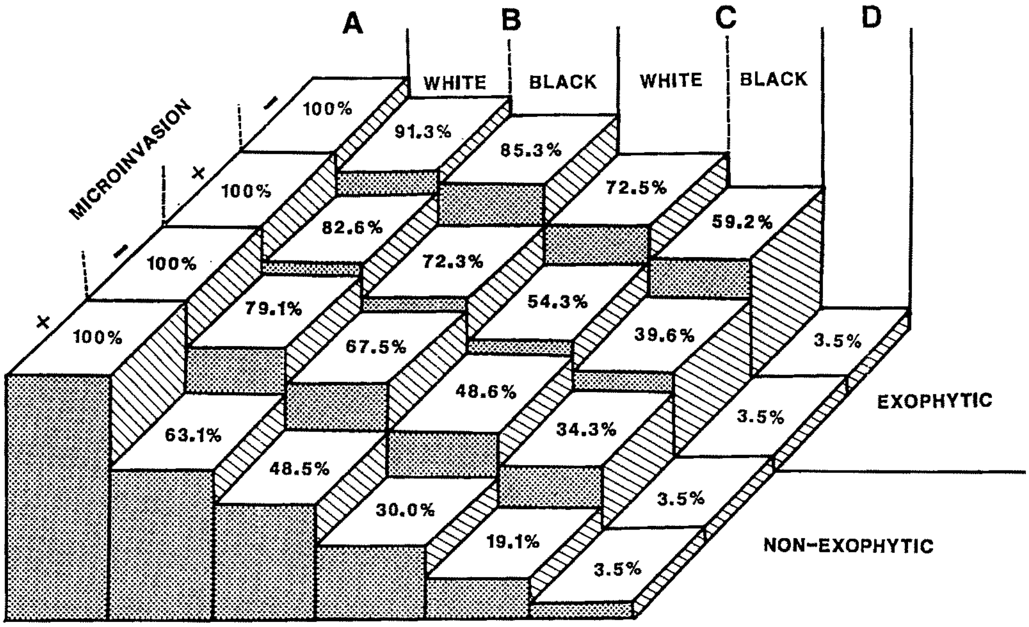


FIG. 5. Estimates of 5-year survival probabilities for colorectal adenocarcinoma. For each of the 16 Dukes' stages B and C subgroups, the estimate is based on the four coefficients obtained from the statistical analysis described in Materials and Methods. The respective 5-year survival rates of colon and rectal patients were averaged to provide probability estimates for Dukes' A and D patients.

sponding stage, our rectal staging system yielded a high degree of correlation in 15 of 16 subgroups of colon patients. The only subgroup with an observed 5-year survival rate significantly higher than the predicted rate was the subgroup with the worse prognosis: blacks with stage C, nonexophytic adenocarcinoma with microinvasion. For that group, the observed value was 36% compared to 14% based on the rectal patients. This finding suggests that rectal carcinoma, characterized by these more severe features, may have a more dismal prognosis than a similarly described colon carcinoma.

Our finding that rectal and colon carcinoma, when stratified by clinical and pathologic characteristics, have a similar prognosis, differs from that reported previously in the literature. Past reports have suggested that survival, based on the original or modified Dukes' classification, after a potentially curable resection for rectal carcinoma, is poorer than that after a similar operation for intra-abdominal colon cancer. Dwight et al.,[5] reporting on 1064 cases of colorectal cancer, found that stage, site of origin, and technical complications independently (using a multivariate analysis) influenced 5-year survival. Specifically their 5-year survival rate after curative resection of colon carcinoma was 61% compared to 43% for rectal carcinoma; while their rates for Dukes' B and C were 65% and less than 37%, respectively. Other authors,[6,7] using univariate analysis, have agreed with Dwight in asserting that site exerts a negative influence in long-term survival.

We believe that the difference in conclusions is due to the multifactorial nature of colorectal adenocarcinoma, and that once certain features are identified and incorporated into the analysis, the issue of the site of the lesio loses its influence on 5-year survival. This hypothesis i supported by the fact that our results, grouped by Dukes stage, indicate a better prognosis for colon patients tha for rectal patients, but when grouped into more homogenous subgroups, the apparent difference disappears. Th discrepancy probably reflects the finding that rectal cancers more frequently than colon cancers have characteristics associated with a worse outcome (*i.e.*, more advanced stage, nonexophytic lesions, and presence of vascular and/or lymphatic microinvasion).

Therefore, to generate a more accurate clinical pathologic staging system for colorectal adenocarcinoma, w combined the data from the rectal patients and colon patients. The staging system is divided into 18 subgroups The first subgroup, stage A, is identical to the stage A o the Astler-Coller modification of Dukes' classification. The last subgroup, stage D, identifies tumors with distan metastases (carcinomatosis, liver, lung, and so on). Th prognosis at 5 years in these two groups is so well define that other clinicopathologic variables have little to contribute in terms of better prognostic accuracy. The remaining patients fall into the 16 subgroups derived fro combining the four significant variables described above Figure 5 graphically displays this new clinicopathologi staging system for colorectal adenocarcinoma, indicatin the 5-year survival rate for each subgroup.

We believe that an improved prognostic capability wil enable surgeons not only to advise their patients mor accurately as to their prognosis but also to identif subgroups of high-risk or low-risk patients. This will allo them to determine the need for adjuvant therapy more reliably, assess its effects more appropriately, and allocate follow-up resources more effectively and efficiently.

Acknowledgments

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A Comparison of Intensive Care Unit Care of Surgical Patients in Teaching and Nonteaching Hospitals

SAMIR M. FAKHRY, M.D., JEFFREY L. BUEHRER, M.D., GEORGE F. SHELDON, M.D.
and ANTHONY A. MEYER, M.D., PH.D

From the Department of Surgery, School of Medicine
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

Three hundred forty-eight teaching (TH) and 282 nonteaching (NTH) hospitals were surveyed to determine how intensive care unit (ICU) care is delivered to surgical patients and current views on surgical critical care. Teaching hospitals were more likely than NTHs to have a separate surgical ICU (92% *versus* 37%), a dedicated ICU service/physician (37% *versus* 7%), and a surgeon as director of the ICU (67% *versus* 29%). All THs and 33% of NTHs provided 24 hour in-house coverage for the ICU. A majority of respondents preferred a surgeon as ICU director (TH, 85%; NTH, 67%) and felt that critical care was an essential part of surgery (THs, 87%; NTHs, 74%). Most (THs, 58%; NTHs, 56%) thought that a cooperative effort between the primary service and an ICU service provided better patient care, but only 37% of THs and 22% of NTHs provided care with such a system. Many (THs, 45%; NTHs, 33%) thought that surgeons are willingly relinquishing ICU care. Surgeons continue to desire responsibility for their patients in the ICU and most prefer ICU service involvement provided by surgeons. This discrepancy between what is practiced and what is desired, along with proposed changes in reimbursement for surgery and the recent definition of critical care as an essential part of surgery, may stimulate greater involvement of surgeons in critical care.

THE CARE OF critically ill patients in the intensive care unit (ICU) is a significant part of modern clinical medicine in all major specialties. Critical care needs account for up to 10% of hospital beds and up to 15% of inpatient health expenditures.[1-3] The increasing complexity in available technology and treatment modalities has placed added demands on practicing surgeons, effectively competing with traditional activities in the operating room, the office, and the wards. Quality patient care requires up-to-date knowledge of critical care and a mechanism to provide this care in a consistent, uninterrupted fashion. The ICU care of surgical patients is delivered in a variety of formats, conforming to the needs and resources of different institutions. The role of surgeons in the care of these critically ill patients has been examined in several recent reports dealing predominantly with the academic setting.[4-6] These reports stated that surgeons should be responsible for the care of their critically ill patients. The American College of Surgeons, the American Board of Surgery (ABS), and the Residency Review Committee are among the professional organizations that stress the importance of the 'complete care of critically ill patients with underlying surgical problems' to include the ICU stay. The ABS considers critical care among the 'essentials' of general surgery[7] and has issued a Certificate of Added Qualification in Surgical Critical Care.

In a recent survey of the 296 program directors of approved General Surgery training programs, 91% though that critical care should be considered one of the 'essentials' of General Surgery and 72% of program directors thought that there should be a separate surgical ICU rotation for general surgery residents.[8] In only 47% of the programs, however, did a designated surgical ICU service exist to provide surgical residents with the necessary training. Although this survey and other reports[4-6,8] provide some insight into the ICU care of surgical patients in teaching hospitals (TH), there is little data available from nonteaching hospitals (NTH) on the ways in which care of critically ill surgical patients is delivered. Similarly little is known of the views of surgeons at NTHs with respect to surgical critical care (SCC). This study was undertaken to compare ICU care of surgical patients at THs and NTHs and the views of surgical leaders at both types of institutions with regard to SCC.

Address reprint requests to Samir M. Fakhry, M.D., Department of Surgery, 167 Burnett-Womack Bldg., CB 7210, University of North Carolina, Chapel Hill, NC 27599-7210.

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Methods

A surgical critical care questionnaire was sent to the program directors and/or chiefs of surgery in all the hospitals in the 296 general surgery teaching programs listed in the *1987–1988 Directory of Graduate Medical Education Programs.*[9] A second questionnaire was sent 4 months later if there was no response to the first request. A similar questionnaire was addressed to the chief of surgery at 282 NTHs randomly selected from the *American Hospital Association's Guide to the Health Care Field.*[10] The questionnaires addressed the program/hospital description; the available hospital and ICU beds; the attending staff; the ICU resources; the conduct of patient care, including coverage and billing; and the views of the program director or chief of surgery with respect to SCC issues. The two questionnaires were nearly identical, the one sent to THs containing additional questions pertaining to resident education and the one sent to the NTHs containing additional questions related to functions of the attending physicians, some of which are assumed by residents in THs. Questions common to both questionnaires were written in an identical manner.

The data were compiled and entered into a microcomputer database for analysis. Answers were expressed as frequencies of replies and rounded to the nearest 1%.

Results

We received responses from 235 of 296 academic programs containing data from 348 THs. Answers from individual THs were considered separately and served as the denominator for THs in the analysis. The response rate by general surgery teaching program was 79% (235 of 296). We received responses from 121 NTHs, for a response rate of 39% (121 of 282) (Table 1).

Critical Care Facilities (Table 2)

The average number of hospital beds at THs was 539 +/− 300 and at the NTHs it was 338 +/− 137. The average number of ICU beds at THs was greater than at NTHs (45 compared to 21), as was the number of surgical ICU beds (17 compared to 9). All hospitals responding had at least one ICU. Ninety-two per cent of THs had a separate surgical ICU while only 37% of NTHs had a separate surgical ICU. The most common type of ICU at the THs was the surgical ICU, followed by coronary care units (CCU) in 81%, medical ICU (MICU) in 80%, neonatal ICU in 56%, and cardiothoracic ICU in 50%. The most common ICU at the NTHs was the combined medical–surgical ICU, present in 69% of NTHs, followed by the CCU present in 28%, the SICU in 37%, and the MICU in 33%. Analysis of trauma center designation also was included. Significantly more THs than NTHs had level 1 designation: 135 (39%) THs compared to 7 (5.8%) NTHs.

TABLE 1. *Survey Responses*

Surveys	TH	NTH
Surveys sent	296 programs	282 hospitals
Surveys received	235 programs 348 hospitals*	121 hospitals
Response rate	79%	43%

* Some programs had more than one teaching hospital and a survey form was included for each individual hospital.

TH, teaching hospital; NTH, nonteaching hospital.

TABLE 2. *Critical Care Facilities*

Facilities	TH	NTH
Average number hospital beds	539 ± 300	338 ± 137
Average number ICU beds	45 ± 28	21 ± 13
Average number surgical ICU beds	17 ± 14	9 ± 4
Hospitals with separate surgical ICU	92%	37%
Most common ICU	Surgical (92%)	Combined med-surg (69%)
Level 1 Trauma Center designation	42%	6%
Level 2 Trauma Center designation	22%	27%
Level 3 Trauma Center designation	7%	7%

TH, teaching hospital; NTH, nonteaching hospital.

Administration (Table 3)

A surgeon was the director or codirector of the surgical ICU in 67% of THs and 40% (93) of these surgeon directors held an ABS Certificate of Added Qualification in Surgical Critical Care. A surgeon served as director or codirector of the ICU in which surgical patients received care in 29% of NTHs and only 12% (4) of these surgeons had the ABS Certificate of Added Qualification in Surgical Critical Care.

Patient Care (Table 3)

A separate surgical ICU service existed in 37% of THs. Only 7% of NTHs had a physician whose sole responsibility was in the ICU. In 33% of NTHs a physician was available on call in the hospital 24 hours a day, while in the remainder, ICU call was from home or by other arrangement. In THs with a designated surgical ICU service, the care of the patient in the ICU was rendered through a cooperative effort between the primary service and the ICU-based service in 63% of hospitals. In THs without a designated SICU service, the primary surgeon was solely responsible for care in 72% of hospitals. At NTHs the primary surgeon was responsible for care in 69% of hospitals, while care was rendered through a cooperative effort between the primary physician and another physician in 22% of hospitals. There was no instance in our sample of NTHs of an ICU physician/service assuming primary responsibility for a patient's ICU care and only 10 THs

TABLE 3. *Administration and Patient Care*

	TH (%)	NTH (%)
Surgeon ICU director	67	29
Director has ABS SCC certificate	40	12
Dedicated SICU service/MD	37	7
24 hour physician coverage		
In house	100	33
In ICU	37	7
Primary responsibility for ICU care provided by		
Primary surgeon	55	69
ICU service/MD	4	—
Cooperative effort	33	2
ICU service/physician participate in		
Ventilator management	51	36
Emergency interventions	48	36
Referring physician participates in postoperative care in ICU		
Always	N/A	5
Frequently	N/A	28
Occasionally	N/A	43
Never	N/A	11

TH, teaching hospital; NTH, nonteaching hospital.

provided ICU care to critically ill surgical patients primarily through the ICU service.

In response to the question to the chief of surgery at NTHs, 'How often do nonsurgical physicians in your hospital expect to provide postoperative critical care management of patients they refer for surgery?' the following answers were received: never, 11%; occasionally, 43%; frequently, 28%; always, 5%; no response, 13%.

When a surgical ICU service existed at a TH, it controlled admissions and discharges in 65% of hospitals and managed ventilators, fluids, and 'drips' in 75%. The surgical ICU service at THs made rounds with the primary service in 67% of cases and provided in-house resident coverage with attending back-up in 92% of hospitals. The ICU physician caring for surgical patients at NTHs controlled admissions and discharges in 16% of hospitals, managed ventilators, fluids, and 'drips' in 20%, and made rounds with the primary service in 7% of hospitals. In-house physicians were available 'on-call' for the ICU in 33% of NTHs.

Economic Considerations

The surgical ICU service in THs billed separately for critical care services in 43% of hospitals with such a service, for individual consultation in 45%, and for procedures in 50%. The physicians designated for the ICU at NTHs in which surgical patients were managed billed for these services at substantially lesser frequency: 10%, 15%, and 12%, respectively.

ICU Bed Availability

A shortage of ICU beds resulted in a limitation being placed on admissions to the ICU and in operative procedures performed in 59% of THs and in 24% of NTHs. When ICU admissions had to be limited, among the reasons cited were nursing shortages (69% of THs and 77% of NTHs), bed availability (76% of THs and 95% of NTHs), and the unwillingness of physicians to transfer noncritically ill patients out of the ICU (18% of THs and 50% of NTHs).

Views (Table 4)

Eighty-seven per cent of chiefs of surgery at THs and 74% of chiefs of surgery at NTHs believed that critical care should be considered one of the essentials of general surgery. Only 3% at THs and 12% at NTHs disagreed. Sixty per cent at both THs and NTHs thought a certificate of Added Qualification in Surgical Critical Care should be offered by the ABS. Forty-five per cent of program directors at THs and 33% of chiefs of surgery at NTHs thought the care of critically ill surgical patients was being willingly relinquished by surgeons. Eighty-five of those at THs would prefer a surgeon as surgical ICU director and 70% thought he or she should be certified in critical care, while 67% at NTHs would prefer a surgeon as surgical ICU director and 55% thought he or she should be certified in critical care. Most respondents in both groups (THs, 57%; NTH, 56%) thought optimal care in the surgical critical care setting would be rendered by a combination of the primary service/physician and the ICU service/physician. Most respondents (71% in THs and 54% in NTHs) would encourage general surgery residents to enroll in critical care training fellowships.

Discussion

Surgeons have traditionally provided the complete spectrum of care to patients with surgical diseases.[10-12] They have been responsible for many major advances in all aspects of patient care and participated in the development of the ICU concept and many of the subsequent innovations and technologies.[13] A rapid expansion in knowledge of normal and abnormal physiology and an exponential growth in the technologies of health care have resulted in added demands on physicians providing care in the critical care setting. As these innovations and tech-

TABLE 4. *Views of Chiefs of Surgery*

View	TH (%)	NTH (%)
Prefer surgeon SICU director	85	67
SCC essential in general surgery	87	74
Cooperative effort between ICU service and primary service provides better patient care	58	56
Best ICU care with cooperative system	37	22
ICU care of surgical patients being willingly relinquished	45	33

TH, teaching hospital; NTH, nonteaching hospital.

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nologies become more elaborate and the therapeutic modalities used in the critical care setting continue to increase in complexity, the practicing surgeon finds it more difficult to maintain a level of proficiency to provide quality care to his or her critically ill patients in the ICU. Surgeons are faced with simultaneous demands in the operating room, the wards, and in the laboratory in academic settings. Sometimes surgeons have found it necessary and, in some cases, convenient to seek the assistance of other physicians in providing portions of the preoperative and postoperative care of patients. As is apparent from our survey, it is not uncommon for the referring physician in the NTH to expect to provide preoperative and postoperative care to patients referred for surgery. Patients in the ICU may require expertise not readily available at the level of the referring physician and thus require the addition of another specialist (often in pulmonary medicine) to the physician roster who generates further charges to the patient. Although a shared, cooperative effort for certain complex problems is practical and may be beneficial, completely relinquishing care is clearly undesirable.

This study shows that there are significant differences in the ICU care of surgical patients at THs and NTHs. This includes differences in both management practices and resource allocation. These differences are due, in part, to the THs being on average larger than the NTHs. The ICUs for surgical patients at THs are also larger than their counterparts in NTHs. There is a higher degree of ICU coverage at THs, with physicians available in-house on a 24-hour basis at all THs but in only 33% of NTHs. A separate surgical ICU service existed at 37% of THs, while few (7%) NTHs had a physician solely designated for the ICU. The quantitative differences are not completely the result of the greater bed capacity of THs because that does not explain the difference in coverage patterns and in direct patient care practices. We assume that the presence of residents at THs accounts for much of the difference in coverage and management practices and that patient acuity also has a significant effect. The surgeon's rapport with the patient's referring physician and other specialists has practical implications at NTHs; our data suggest that in up to 75% of cases the surgeon is under some pressure to allow others to provide postoperative care. Our data do not allow us to determine the relative contribution of such factors.

Differences in management practices also were noted. A designated surgical ICU service existed at 37% of THs while only 7% of NTHs had a physician designated solely for the ICU in which surgical patients were located. A separate surgical ICU existed in 92% of THs and was the most common type of ICU at the academic institutions. Only 37% of NTHs had a separate surgical ICU and the most common type of ICU there was the combined medical–surgical unit. This arrangement and the paucity of surgeon-directors at NTH underscores the shortage of surgeons interested and qualified to administer ICUs in the nonacademic setting. It has been suggested that some surgeons may find it more convenient and remunerative to operate while someone else provides ICU care and administration.[6,14] Of practical significance, there are more than 3000 internists (predominantly pulmonologists) and anesthesiologists holding critical care certificates from their respective board. As of August 1990, the number of surgeons holding a Certificate of Added Qualification in Surgical Critical Care from the ABS is approximately 508. This is the largest number of any specialty except internal medicine.

While the manner in which ICU care for surgical patients is administered differs significantly between THs and NTHs, the views of surgical leaders at both types of institutions are similar (Table 4). The majority of respondents at both THs and NTHs thought that critical care was an essential part of general surgery and that a surgeon should direct the ICU where surgical patients are treated. More than one half the respondents at both THs and NTHs thought that a cooperative effort between the primary surgeon and an ICU service/physician provided optimal care, but only a minority at both types of hospitals provided care with such a system. A significant number of these leaders thought critical care was being willingly relinquished by surgeons.

The views expressed by the respondents are in accord with the frequent assertion that surgeons provide postoperative care for their patients in the critical care setting and that they retain control of their ICUs.[4,6,7,10,11,14] The manner in which ICU care is presently provided, however, suggests that the implementation of these ideals is less than desired. This is true at both THs and NTHs, although the situation at NTHs is more pronounced. In a study of ICU care of surgical patients in the private practice environment, Trask and Faber[15] reached similar conclusions. Their survey of the chiefs of surgery and the nurse managers of ICUs at 188 private hospitals revealed that 'surgeons did not have the principal managing role in the ICU in 70% to 75% of the hospitals' and that 'surgeons are relinquishing their responsibilities in the direct care of the preoperative and postoperative critically ill patients.' They suggest that there are three main reasons for this: the complexity of critical care, a lack of economic incentives, and professional liability.

Resolution of these issues may be influenced by forces from within organized surgery as well as external factors. On the one hand, surgeons and their professional organizations have begun to respond to the growing need for trained surgeons to direct ICUs. The ABS has asserted that critical care is an essential part of general surgery and provided a mechanism for credentialing qualified surgeons in SCC. Fellowship programs in critical care are offered by 93 institutions in the United States.[16] Of these approximately 22 are based in academic surgical departments. At present, only five are approved by the Residency Review Committee for Surgery. Proposed changes in reimbursement for surgical services, such as the global fee concept, may provide further impetus for change. If procedure fees included postoperative care, the operating surgeon would be expected to provide ICU care and would therefore need to be reasonably proficient in such care whether in THs or NTHs.

The impact of a designated ICU physician on the care of critically ill patients has been studied. Li et al.[17] reported that the introduction of a designated ICU team resulted in improved ICU and in-hospital outcomes in a community hospital. This was accompanied by a significant increase in the use of invasive monitoring and blood tests and a shorter ICU stay for lower-risk patients. Knaus et al.[18] reported that the degree of coordination of ICU care significantly influenced its effectiveness. Therefore there is a role for qualified surgeons to direct surgical ICUs, provide on-site availability, and manage resource allocation and quality assurance.

Our data suggest that most chiefs of surgery at both THs and NTHs endorse a system whereby surgeons control their ICUs and provide care for their critically ill patients. The actual mechanisms by which care is delivered suggests that there is a shortage of qualified surgeons to direct ICUs and manage critically ill surgical patients. Furthermore it appears that general surgeons are often unwilling or unable to provide ICU care, particularly at NTHs. In the present reimbursement climate, the incentives and referral patterns, especially at NTHs, favor a lesser level of involvement of surgeons in ICU care. General surgery training programs may influence these trends by establishing SCC services, maintaining control of their ICUs, and sponsoring fellowships in SCC. Such action would result in continued emphasis on the training of general surgeons in SCC and in providing a pool of qualified surgeons who are credentialed in SCC to administe and manage surgical ICUs. It is probable that in the absence of such efforts, surgeons may continue to relinquis their role in the ICU.

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Differential Pathophysiology of Bacterial Translocation After Thermal Injury and Sepsis

WILLIAM G. JONES II, M.D., ANNABEL E. BARBER, M.D., JOSEPH P. MINEI, M.D., THOMAS J. FAHEY III, M.D., G. TOM SHIRES III, M.D., and G. TOM SHIRES, M.D.

Bacterial translocation (BT) occurs transiently after thermal injury and may result from an ischemic intestinal insult. To evaluate continued intestinal ischemia in the ongoing BT associated with sepsis after injury, rats were randomized to (1) 30% burn injury with *Pseudomonas* wound infection (BI), (2) BI + fluid resuscitation (BI + Fluid), (3) BI after allopurinol pretreatment to inhibit xanthine oxidase (BI + Allo), or (4) BI after azapropazone pretreatment to inhibit neutrophil degranulation (BI + Aza). On postburn days (PBD) 1, 4, and 7, animals were studied for evidence of BT and intestinal lipid peroxidation. BI + Fluid, BI + Allo, and BI + Aza significantly ($p < 0.05$) reduced rates of BT and ileal lipid peroxidation acutely after thermal injury (PBD 1) compared to BI. All four groups had equally high rates of BT associated with the onset of sepsis (PBDs 4 and 7), without evidence of further intestinal lipid peroxidation. These data indicate that the chronic gut barrier failure associated with sepsis after injury occurs independently of continued intestinal ischemia.

From the Department of Surgery, Cornell University Medical College, New York, New York

INCREASING EVIDENCE SUGGESTS that failure of the gastrointestinal tract to function as an effective barrier against the absorption of intraluminal bacteria and endotoxin may participate in both the acute and chronic responses to systemic injury. Bacterial translocation in animal model[1-3] and increased intestinal permeability to lactulose in humans[4,5] have been used as experimental markers of such gut barrier dysfunction. These markers increase dramatically after acute thermal injury and diminish with recovery.[2]

Gut barrier dysfunction after acute injury may result from an ischemic insult to the intestinal mucosa. Thermal injury in animal models is accompanied by an acute decrease in splanchnic blood flow occurring within hours after injury, with return to baseline levels by 24 hours after burn.[6,7] Bacterial translocation in animal models after thermal injury may be diminished significantly by treatment with mesenteric arterial infusion of vasodilators[8] or by pretreatment with angiotensin-converting enzyme or xanthine oxidase inhibitors.[7,9] It is thus postulated that hypovolemia and splanchnic vasoconstriction after cutaneous thermal injury lead to relative intestinal ischemia, with gut barrier damage produced by activation of tissue enzymes and neutrophil degranulation.

Following uncomplicated injury, gut barrier failure is a transient event, with restoration of normal function occurring within several days.[1,10] However conversion to a chronic injury state by the superimposition of infection after thermal injury leads to prolonged and more pronounced gut barrier dysfunction. In humans increased intestinal permeability to lactulose is significantly prolonged in infected burned patients as opposed to those whose clinical courses are uncomplicated by infection.[4] In rats translocating enteric organisms, present in the mesenteric lymph nodes on postburn day 1, are cleared by the fourth postburn day after uncomplicated injury, but persist through the seventh postburn day after thermal injury and infection.[10] During burn wound sepsis, translocating organisms also may progress beyond the mesenteric lymphatics to the abdominal viscera and ultimately into the bloodstream.

Previously we demonstrated that splanchnic blood flow, as determined by ^{51}Cr-labeled microspheres, is maintained at baseline levels during burn wound sepsis in rats,[11] leading us to question the role of continued intestinal ischemia in the etiology of the prolonged gut barrier failure seen

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Address reprint requests to William G. Jones II, M.D., Department of Surgery, Room F-739, The New York Hospital—Cornell Medical Center, 525 East 68th St., New York, NY 10021.

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with sepsis. While the total blood flow to the intestine appeared unchanged during burn wound sepsis, this methodology does not eliminate the possibility of intramural shunting of blood flow within the intestine resulting in mucosal ischemic injury. The purpose of the present study was thus to further evaluate whether chronic intestinal ischemia participates in the etiology of prolonged gut barrier dysfunction during sepsis after injury. To accomplish this objective, we sought to prevent intestinal ischemic injury in a model of thermal injury and subsequent burn wound sepsis through maintenance of adequate intravascular volume *via* aggressive fluid resuscitation, and inhibition of xanthine oxidase activation and neutrophil degranulation by allopurinol or azapropazone pretreatment, respectively.

Methods

Study Design

Male Wistar rats (weighing 310 ± 25 g) were randomized to (1) 30% scald burn injury with wound inoculation with 1×10^8 colony-forming units of *Pseudomonas aeruginosa* (BI); (2) burn injury and infection as above followed by fluid resuscitation with lactated Ringer's solution (0.5 mL/percentage burn/day) by intraperitoneal injection (BI + Fluid); (3) 3-day pretreatment with the xanthine oxidase inhibitor allopurinol (50 mg/kg/day; Zyloprim®, Burroughs-Welcome, Research Triangle Park, NC) by gavage followed by burn injury and infection as above (BI + Allo); or (4) 5-day pretreatment with the anti-inflammatory agent azapropazone (100 mg/kg/day; Du Pont Pharmaceuticals, Wilmington, DE) by gavage followed by burn injury and infection as above (BI + Aza). Fluid, allopurinol, and azapropazone treatments were continued after burn through the conclusion of the study period.

Body weight was recorded daily and on postburn days 1, 4, and 7 animals were killed by anesthetic overdose (n = 12 animals/group/time point) and cultures obtained of the mesenteric lymph nodes, abdominal viscera, blood and cecal contents for evidence of bacterial translocation. Peritoneal swab cultures also were obtained from the B + I + Fluid group, which had received intraperitoneal injections after burn. Caval blood samples were obtained from the B + I and B + I + Fluid groups for hematocrit determinations, and the small bowels, livers, and spleens from these groups were excised for gravimetric determinations of total mass and tissue water content. Segments of terminal ileum were obtained from all animals for assay of tissue byproducts of lipid peroxidation.

Burn and Infection Model

A 30% dorsal scald burn was produced by immersion in 95 C water for 10 seconds using a plastic template after induction of adequate general anesthesia by intraperitoneal pentobarbital, as described by Walker and Mason.[12] After burn injury, wounds were inoculated immediately with 1×10^8 *Pseudomonas aeruginosa* strain PSA 59-1244, as described by Yurt.[13] This model previously was shown to result in onset of sepsis by postburn day 4, as evidenced by the clinical signs of anorexia, conjunctival hemorrhage, piloerection, and hypothermia.[10,11,14,15] A prolonged counter-regulatory hormone response occurs,[14] and 100% mortality results within 14 days after burn.[15]

Determination of Bacterial Translocation

After anesthetic overdose, the ventral skin was cleansed with 70% isopropyl alcohol and the abdominal cavity opened. One hundred-milligram specimens of the mesenteric lymph nodes, liver, spleen, and 0.5 mL caval blood were collected using sterile techniques. Specimens were then placed in 5-mL sterile brain–heart infusion (BHI) broth and homogenized with sterile teflon-coated tissue grinding rods (Cat. No. 08-414-14D, Fisher Scientific, Pittsburgh, PA), using a modification of the technique of Berg.[16] Two hundred-microliter aliquots of each tissue homogenate were then plated on each of sheep blood and MacConkey's agar culture plates. One half-milliliter specimens of the cecal contents were similarly obtained, placed in 10-mL BHI broth, homogenized, and serially diluted in sterile saline. Aliquots of each dilution also were plated as above. All cultures were incubated at 37 C and examined for growth at 24 and 48 hours, with organisms identified by standard bacteriologic techniques. Growth of any colony-forming units of enteric bacteria on either medium was considered a positive culture.

Determination of Intestinal Content of Lipid Peroxidation Byproducts (Malondialdehyde)

One hundred-milligram segments of terminal ileum were obtained and immediately frozen in liquid nitrogen and stored at −70 C until analysis. Samples subsequently were homogenized in buffer and assayed colorimetrically for malondialdehyde (MDA) content using the thiobarbituric acid reaction as described by Ohkawa et al.[17] Briefly, 10% weight/volume ileal homogenate was mixed with sodium dodecyl sulfate and aqueous thiobarbituric acid, while pH was maintained at 3.5 with acetate buffer. Specimens were placed in a boiling water bath for 1 hour and the resultant red pigment extracted with *n*-butanol-pyridine and estimated by the spectrophotometric absorbance at 532 nm against a tetramethoxypropane standard. Malondialdehyde content was then expressed as nmol/g tissue.

Statistical Analysis

Data is reported as the mean ± standard error of the mean (SEM) where appropriate. Comparisons of the incidence of positive cultures were made using chi square analysis. Comparisons of weight data and quantitative culture results were made with one-way analysis of variance (ANOVA) with Newman-Keuls multiple range testing.

Results

Figure 1 depicts changes in body weight during the study period. As previously reported,[10,11,14] the combination of burn injury and infection results in marked cachexia, as demonstrated by the 27% cumulative weight loss seen in the BI group by postburn day 7. Neither treatment with allopurinol nor azapropazone significantly prevented the body wasting associated with this model of burn wound sepsis. However, despite food intake similar to that of the other groups, animals receiving fluid resuscitation of 15 mL/day (0.5 mL/percent burn/day) had an attenuated weight loss ($p < 0.05$ *versus* all groups on postburn days 4 and 7). This occurred in association with maintenance of normal hematocrit values, as opposed to BI animals, which demonstrated significant hemoconcentration (Fig. 2).

Burn wound sepsis is accompanied by intestinal atrophy with a significant decrement of small bowel mass by postburn day 7.[10,11] Intraperitoneal administration of fluids in this model, however, resulted in increased small bowel mass on postburn day 7 compared to the unresuscitated groups, even though a similar initial loss of intestinal mass was observed in all groups (Fig. 3). Both hepatic and splenic mass also were significantly elevated in the BI + Fluid group compared to BI on postburn day 7, (liver: 6.84 ± 0.33 *versus* 5.65 ± 0.31; spleen 0.75 ± 0.05 *versus* 0.49 ± 0.03; $p < 0.01$ in each case). The increased organ weights in the resuscitated group appear to represent the accumulation of edema fluid within the tissues, as evidenced by a significantly increased small bowel water content (Fig. 4). Small bowel mass, but not hepatic or splenic mass, also was noted to increase in azapropazone-treated animals and may represent some local action of this drug.

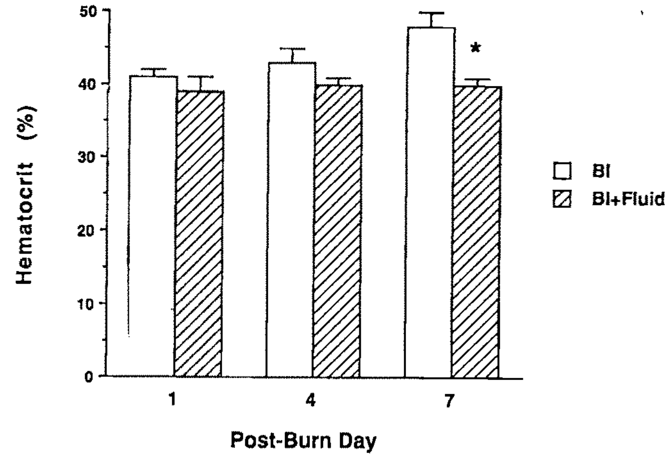

FIG. 2. Hematocrit values (mean ± SEM) during the 7-day study period. Significant hemoconcentration was evident in the unresuscitated BI group on postburn day 7 (*$p < 0.05$).

The incidence of bacterial translocation to the mesenteric lymph nodes (MLN), abdominal viscera, and blood in all groups is shown in Table 1. The administration of intraperitoneal fluid resuscitation, or treatment with either allopurinol or azapropazone, significantly re-

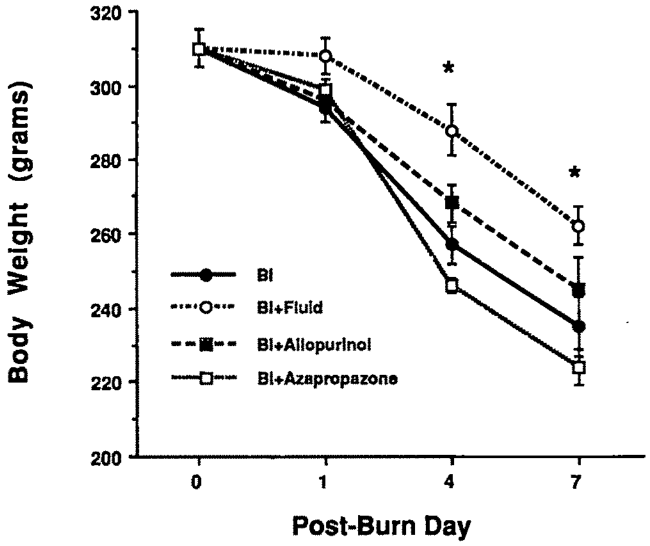

FIG. 1. Body weight change (mean ± SEM) during the 7-day study period. The BI + fluid group suffered significantly less cachexia and weight loss than all other groups (*$p < 0.05$).

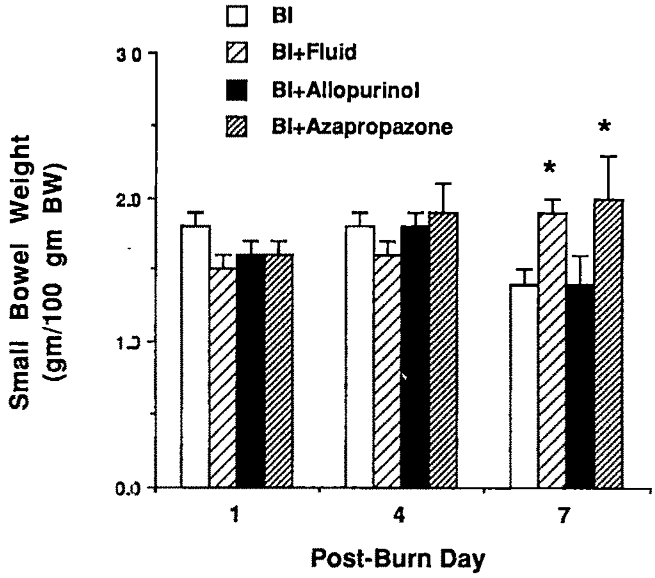

FIG. 3. Small bowel weight normalized for body weight during the 7-day study period. Significant increases in small bowel mass (mean ± SEM) were noted in the BI + fluid and BI + azapropazone groups on postburn day 7 (*$p < 0.05$).

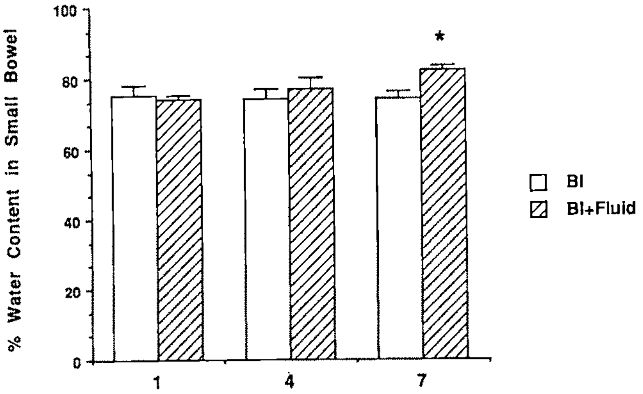

FIG. 4. Small bowel water content during the 7-day study period. Significant bowel edema, evidenced by an increase small bowel water content (mean ± SEM) was evident in the BI + fluid group by postburn day 7 (*$p < 0.05$).

duced the incidence of bacterial translocation to the MLN on postburn day 1. The abdominal viscera and blood remained sterile at this time in all groups, as in our previous report of translocation in this model.[10] However none of these regimens significantly impacted on the subsequent rates of translocation to the MLN, liver, spleen, or blood after the onset of sepsis, (postburn days 4 and 7). In all groups the predominant translocating organism was *Escherichia coli*, with the occasional appearance of other gram-negative species such as *Proteus mirabilis* and *Klebsiella pneumoniae.*

The results of quantitative cultures of the MLN, cecal flora, and burn wounds from each group are shown in Table 2. Both allopurinol and azapropazone treatment were associated with a significantly ($p < 0.05$) reduced number of enteric organisms per gram MLN compared to the BI and BI + Fluid groups on postburn day 1. No significant differences in this parameter were noted on either of the other study days. A significant decrease in the content of gram-negative facultative aerobes in the cecal flora was produced by both allopurinol and azapropazone administration, but these alterations did not correlate with either the incidence of bacterial translocation or the number of enteric organisms present per gram MLN tissue. None of the regimens had any impact on the growth of *Pseudomonas* in the burn wound.

TABLE 1. *Bacterial Translocation of Enteric Organisms After Thermal Injury and Infection in Each Treatment Group*

Group	MLN	Liver	Spleen	Blood
Day 1				
BI	10/12*	0/12	0/12	0/12
BI + fluid	3/12	0/12	0/12	0/12
BI + allo	2/12	0/12	0/12	0/12
BI + aza	3/12	0/12	0/12	0/12
Day 4				
BI	12/12	11/12	11/12	0/12
BI + fluid	10/12	10/12	10/12	0/12
BI + allo	12/12	12/12	12/12	0/12
BI + aza	10/12	10/12	10/12	0/12
Day 7				
BI	12/12	11/12	10/12	5/12
BI + fluid	12/12	10/12	11/12	3/12
BI + allo	12/12	12/12	8/12	5/12
BI + aza	12/12	12/12	12/12	4/12

MLN, mesenteric lymph nodes; BI, burn injury and infection.

Data = positive cultures/n; translocating organisms included *E. coli, P. mirabilis,* and *K. pneumoniae.*

* $p < 0.05$ *vs.* all groups by chi square/Yates analysis.

TABLE 2. *Results of Quantitative Cultures of the Mesenteric Lymph Nodes, Cecal Contents, and Burn Wound Biopsies in Each Treatment Group*

Group	MLN	Cecal Contents†	Burn Wound‡
Day 1			
BI	206 ± 16	$6.4 \pm 0.8 \times 10^8$	$7.1 \pm 1.0 \times 10^6$
BI + fluid	250 ± 35	$3.1 \pm 2.2 \times 10^8$	$8.0 \pm 2.0 \times 10^6$
BI + allo	123 ± 13*	$2.3 \pm 1.5 \times 10^{6*}$	$1.0 \pm 0.5 \times 10^6$
BI + aza	91 ± 27*	$9.1 \pm 4.6 \times 10^{7*}$	$3.8 \pm 1.3 \times 10^6$
Day 4			
BI	1466 ± 155	$9.4 \pm 1.7 \times 10^8$	$5.9 \pm 0.5 \times 10^9$
BI + fluid	807 ± 216	$2.1 \pm 0.7 \times 10^8$	$5.9 \pm 1.3 \times 10^9$
BI + allo	1285 ± 197	$1.7 \pm 1.5 \times 10^{7*}$	$4.3 \pm 0.7 \times 10^9$
BI + aza	714 ± 297	$5.4 \pm 1.5 \times 10^8$	$5.6 \pm 2.1 \times 10^9$
Day 7			
BI	916 ± 289	$1.3 \pm 0.2 \times 10^9$	$7.1 \pm 0.3 \times 10^9$
BI + fluid	1444 ± 602	$1.7 \pm 1.0 \times 10^{8*}$	$6.6 \pm 0.6 \times 10^9$
BI + allo	1390 ± 242	$7.2 \pm 5.3 \times 10^{6*}$	$3.2 \pm 0.7 \times 10^9$
BI + aza	875 ± 260	$2.2 \pm 0.5 \times 10^{8*}$	$6.3 \pm 1.2 \times 10^9$

MLN, mesenteric lymph nodes; BI, burn injury and infection.

Data = mean ± SEM colony-forming units/g.

* $p < 0.05$ *vs.* BI by ANOVA/Newman-Keuls.

† Numbers of facultative gram-negative bacteria are shown.

‡ Pseudomonas aeruginosa was the sole organism cultured from the burn wounds of all groups at these time points.

Ileal content of the secondary lipid peroxidation byproduct MDA is shown in Figure 5. Accumulation of MDA in the bowel was significantly reduced by allopurinol treatment on postburn day 1 ($p < 0.05$) and reduced to an even greater extent by fluid resuscitation and azapropazone treatment ($p < 0.01$). However no increase in ileal MDA content occurred in the animals in any of these four burned and infected groups on postburn days 4 and 7, implying an absence of lipid peroxidation activity within the intestine at these times.

Discussion

Failure of the gastrointestinal tract to act as an adequate barrier against the absorption of intraluminal bacteria and toxins as a consequence of systemic stress has been proposed as a potential source of morbidity after thermal injury.[18] Evidence of gut barrier failure has been demonstrated in both animals and humans after cutaneous burns.[1,4-10] Bacterial translocation, the passage of viable enteric bacteria from the intact gastrointestinal tract to the mesenteric lymphatics and beyond,[16] has been widely

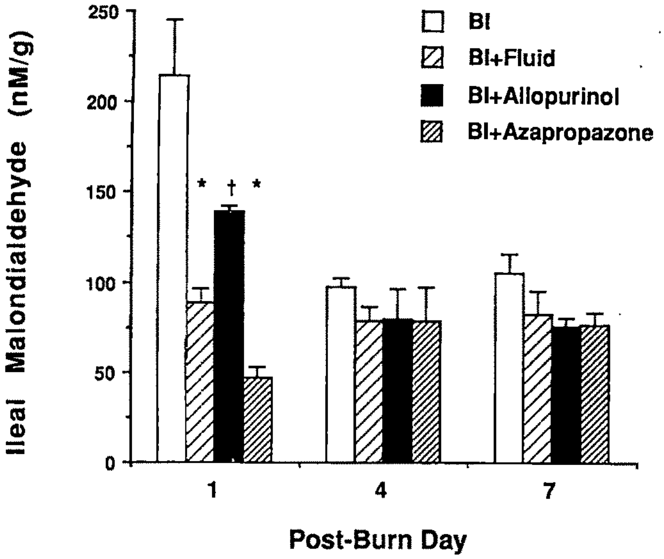

FIG. 5. Ileal malondialdehyde (MDA) content during the 7-day study period. Ileal MDA levels (mean ± SEM) doubled in the BI group on postburn day 1 compared to baseline. This initial increase in lipid peroxidation byproducts was attenuated by fluid ($*p < 0.01$), allopurinol ($\dagger p < 0.05$), and azapropazone ($*p < 0.01$) treatments. Ileal MDA content was not increased in any group on postburn days 4 and 7, despite a high incidence of bacterial translocation.

used as a marker of gut barrier dysfunction in experimental models and has been shown to occur after hemorrhagic shock, endotoxin challenge, and dietary and intestinal flora manipulations, as well as after thermal injury.[1-3,16,19] Gut barrier dysfunction, resulting in bacterial translocation, has been associated with altered regional and systemic responses to subsequent stress[19,20] and may contribute to postinjury hypermetabolism and multiple-organ failure.[21]

Experimental evidence has suggested that gut barrier failure after acute injury may occur as a result of an ischemic insult to the intestine. Intestinal blood flow may be influenced by circulating levels of vasoactive substances such as catecholamines and angiotensin, which are rapidly elevated after thermal injury.[22] Intestinal perfusion then may be further impaired by hypovolemia resulting from fluid sequestration in injured tissues. In animal models, a 50% to 75% increase in splanchnic vascular resistance occurs within 8 hours after a 40% total body surface area full-thickness thermal injury in mini pigs, in association with a 35% decrease in blood flow through the superior mesenteric artery.[23] Reductions in intestinal blood flow of 43% and 46% also have been demonstrated in sheep[6] and rats,[7] respectively, after similar injuries. Maintenance of splanchnic blood flow through pretreatment with an angiotensin-converting enzyme inhibitor[7] or intra-arterial infusion of a vasodilator[8] results in a significantly reduced incidence of bacterial translocation in these models. Furthermore rates of bacterial translocation after thermal injury also may be reduced by pretreatment with xanthine oxidase inhibitors such as allopurinol or tungsten.[9] Thus relative intestinal ischemia appears to be clearly operative in the etiology of bacterial translocation acutely after thermal injury.

Gut barrier dysfunction and bacterial translocation appear to be transient events after thermal injury. In Wistar rats, a 30% scald injury is associated with a 75% incidence of enteric organisms in cultures of the mesenteric lymph nodes on postburn day 1, but the nodes are again sterile by the fourth postburn day.[10] Similarly the number of enteric organisms present in the mesenteric lymph nodes of mice following *E. coli* monoassociation and 30% scald injury decreases 1000-fold between postburn days 1 and 3.[24] These findings imply that repair of the ischemic injury to the gut barrier occurs rapidly after thermal injury.

In contrast the superimposition of a source of infection after thermal injury is associated with prolonged evidence of gut barrier dysfunction. Wistar rats subjected to 30% scald injury and wound inoculation with *Pseudomonas aeruginosa* have similar rates of bacterial translocation on postburn day 1 as animal receiving thermal injury alone, as demonstrated by the BI group in the present study. However infected animals appear to be unable to repair gut barrier damage effectively and clear translocated organisms. Ileal mucosal thickness[10] and synthetic capabilities[11] are severely diminished in burned and infected animals compared to burned or starved animals. A chronic state of gut barrier dysfunction results, leading to an increased incidence of positive mesenteric lymph node cultures by postburn day 4 in infected animals, with a progression of translocated enteric organisms to the abdominal viscera and ultimately into the blood stream not seen after uncomplicated thermal injury.[10]

We sought to determine the role of intestinal ischemia in the etiology of the prolonged gut barrier dysfunction seen in thermal injury complicated by infection. Previously we demonstrated in a model identical to the current BI group that intestinal blood flow, as assessed by ^{51}Cr-labeled microspheres, is maintained at normal levels 3 and 7 days after thermal injury. This finding implies that continued intestinal ischemia is not involved in the etiology of the prolonged gut barrier failure associated with sepsis. However this methodology, while effective at measuring total blood flow to the intestine, does not eliminate the possibility of local ischemia resulting from intramural shunting of blood away from the intestinal mucosa at the villous tips. Thus the three treatment regimens used in the current study were designed to examine further the role of ischemic damage in gut barrier dysfunction during sepsis.

Because fluid sequestration in injured tissues may reduce splanchnic perfusion through intravascular volume depletion and release of vasoconstrictors such as catecholamines and angiotensin, fluid replacement is a logical measure to prevent intestinal ischemia after injury. A standard replacement formula for animal models was used to calculate the estimated fluid requirement, given by intraperitoneal injection.[13] Adequacy of resuscitation was demonstrated by the maintenance of normal hematocrit values throughout the study period in the BI + Fluid group (Fig. 2), while significant hemoconcentration occurred in the unresuscitated BI group. Accumulation of edema fluid in tissues (Figs. 3 and 4) is similar to that clinically observed in septic burned patients. Tissue damage after ischemic insult results largely from the generation of oxygen free radicals and peroxidases. Allopurinol, through inhibition of xanthine oxidase, reduces oxygen radical formation during ischemia and reperfusion injuries and previously was shown to reduce bacterial translocation after uncomplicated thermal injury.[9] Azapropazone is an anti-inflammatory agent shown to inhibit neutrophil migration, aggregation, and degranulation,[25] and thus reduces both free radical generation as well as direct tissue damage by lipid peroxidation.

The effectiveness of these treatment regimens at reducing the initial intestinal ischemic damage after thermal injury is demonstrated by both the reduced rate of bacterial translocation (Table 1) and the prevention of accumulation of lipid peroxidation byproducts in intestinal tissues (Fig. 5) on the first postburn day. However neither fluid resuscitation nor inhibition of xanthine oxidase or neutrophil degranulation had any impact on the prolonged gut barrier failure associated with the onset of sepsis, as evidenced by the high rates of bacterial translocation on postburn days 4 and 7. The systemic progression of translocating organisms probably reflects alterations in host defenses attendant with the development of sepsis. Ileal MDA content, after an initial increase on postburn day 1 in the untreated BI group, returned to baseline levels in this group on postburn days 4 and 7. No significant elevations in ileal MDA were noted in the treatment groups on postburn days 4 and 7 despite a high incidence of bacterial translocation. Therefore these data indicate that the prolonged gut barrier failure associated with sepsis after acute injury in this model appears to occur independently of continued intestinal ischemic damage. Failure to eliminate translocated bacteria due to host immunosuppression, as suggested by Penn et al.,[26] also may contribute to the prolonged presence of enteric organisms in the MLN and to the systemic progression of translocated bacteria.

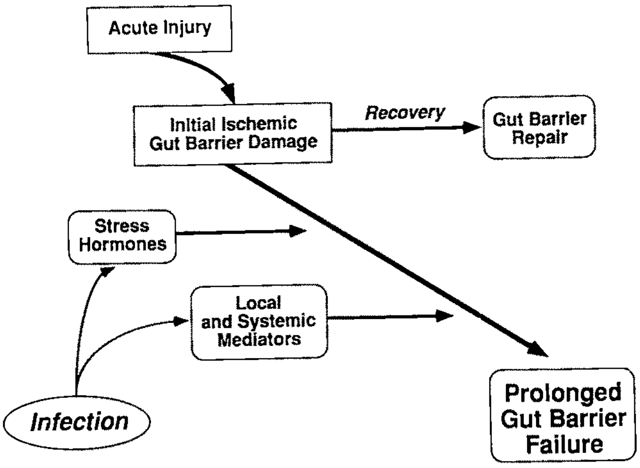
Acute Injury
Initial Ischemic Gut Barrier Damage
Recovery
Gut Barrier Repair
Stress Hormones
Local and Systemic Mediators
Infection
Prolonged Gut Barrier Failure

FIG. 6. Proposed pathophysiology of gut barrier failure after acute injury, and injury complicated by subsequent infection.

Acute thermal injury results in a constellation of systemic host responses that produce effects on body tissues far removed from the area of injury. Host responses such as the production and release of inflammatory and vasoactive mediators occur transiently after simple injury and may contribute to regional alterations in perfusion, relative intestinal ischemia, and the development of gut barrier failure with bacterial translocation. In the absence of complications after acute injury, damage to the gut barrier is quickly repaired and translocated organisms are cleared (Fig. 6). However acute responses to injury may be sustained and exaggerated by the subsequent superimposition of a focus of infection, such as may occur during burn wound sepsis, interfering with repair processes and leading to further gut barrier dysfunction with continued bacterial translocation.

Acknowledgments

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TORADOL® IM (ketorolac tromethamine)

Brief Summary

TORADOL is a nonsteroidal anti-inflammatory drug (NSAID). TORADOL 15 mg/mL solution contains 10% (w/v) alcohol, USP, and 6.68 mg sodium chloride in sterile water. The 30 mg/mL solution contains 10% (w/v) alcohol, USP, and 4.35 mg sodium chloride in sterile water. The pH is adjusted with sodium hydroxide or hydrochloric acid. **Indications and Usage:** Indicated for the short-term management of pain. Not recommended for use as an obstetrical preoperative medication or for obstetrical analgesia because it has not been adequately studied and because of the known effects of NSAIDs on uterine contraction and fetal circulation. Not recommended for routine use with other NSAIDs because of the potential for additive side effects. TORADOL protein-binding is affected by aspirin but not by acetaminophen, ibuprofen, naproxen or piroxicam. Studies with other NSAIDs have not been done. Has been used concomitantly with morphine and meperidine without adverse interactions. **Contraindications:** Do not use in patients with hypersensitivity to ketorolac or with the complete or partial syndrome of nasal polyps, angioedema, and bronchospastic reactivity to aspirin or other NSAIDs. **Warnings:** Although TORADOL Injection is recommended for short-term use only, long-term administration of oral ketorolac has shown that this drug shares the risks of other NSAIDs when taken chronically. Serious GI toxicity, such as bleeding, ulceration, and perforation, can occur at any time, with or without warning symptoms, in patients treated chronically with NSAIDs. Remain alert for ulceration and bleeding in such patients, even in the absence of previous GI tract symptoms. In clinical trials, symptomatic upper GI ulcers, gross bleeding or perforation appear to occur in approximately 1% of patients treated for 3–6 months, and in about 2–4% of patients treated for 1 year. Inform patients about the signs and/or symptoms of serious GI toxicity and what steps to take if they occur. Studies have not identified any subset of patients not at risk of developing peptic ulceration and bleeding. Except for a prior history of serious GI events, and other risk factors known to be associated with peptic ulcer disease, such as alcoholism, smoking, etc., no risk factors (e.g., age, sex) have been associated with increased risk. Elderly or debilitated patients seem to tolerate ulceration or bleeding less well than others and most spontaneous reports of fatal GI events are in this population. High doses of an NSAID probably carry a greater risk of these reactions. In considering the use of relatively large doses (within the recommended dosage range), sufficient benefit should be anticipated to offset the potential increased risk of GI toxicity. **Precautions:** Impaired Renal or Hepatic Function: As with other NSAIDs, use with caution in patients with impaired renal or hepatic function, or a history of kidney or liver disease. Renal Effects: As with other NSAIDs, long-term administration to animals resulted in renal papillary necrosis and other abnormal renal pathology. In humans, hematuria and proteinuria have occurred in long-term trials with oral ketorolac with a frequency and degree similar to aspirin. A second form of renal toxicity has been seen in patients with conditions leading to a reduction in blood volume and/or renal blood flow. In these patients, an NSAID may precipitate overt renal failure. Patients at greatest risk are those with impaired renal function, heart failure, liver dysfunction, taking diuretics, and the elderly. Discontinuation of the NSAID is typically followed by recovery. Use with caution in patients with impaired renal function as reduced creatinine clearance results in reduced clearance of the drug. Follow such patients closely. Fluid Retention and Edema: These have been reported with NSAIDs; use TORADOL with caution in patients with cardiac decompensation, hypertension, or similar conditions. Hepatic Effects: With NSAIDs, borderline elevations of liver tests may occur in up to 15% of patients. These may progress, remain unchanged, or disappear with continued therapy. Elevations of ALT (SGPT) or AST (SGOT) occurred in clinical trials with oral ketorolac in less than 1% of patients. Evaluate a patient with symptoms and/or signs suggesting liver dysfunction, or in whom an abnormal liver test has occurred, for evidence of a more severe hepatic reaction. Hematologic Effects: TORADOL inhibits platelet aggregation and may prolong bleeding time but does not affect platelet count, prothrombin time (PT) or partial thromboplastin time (PTT). Carefully observe patients with coagulation disorders or who are receiving drug therapy that interferes with hemostasis. Inhibition of platelet function by TORADOL disappears within 24–48 hours after the drug is discontinued. In clinical studies, the incidence of clinically significant postoperative bleeding was 0.4% compared to 0.2% in the groups receiving opiates. *Drug Interactions:* TORADOL is highly bound to plasma protein (mean 99.2%), independent of concentration. *In vitro* binding of warfarin to plasma proteins is slightly reduced by TORADOL (99.5% control vs 99.3% with TORADOL 5–10 μg/mL). TORADOL does not alter digoxin protein binding. At therapeutic concentrations of salicylate (300 μg/mL), *in vitro* binding of TORADOL was reduced from 99.2% to 97.5%, a potential 2-fold increase in unbound TORADOL plasma levels; hence, use TORADOL with caution (or at a reduced dosage) in patients being treated with high dose salicylate regimens. Therapeutic concentrations of digoxin, warfarin, ibuprofen, naproxen, acetaminophen, phenytoin, tolbutamide and piroxicam did not alter TORADOL protein binding. In a study of 12 healthy volunteers given TORADOL 10 mg orally for 6 days prior to co-administration of a single dose of warfarin 25 mg, no significant changes in pharmacokinetics or pharmacodynamics of warfarin were detected. In another study of 12 healthy volunteers, co-administration of heparin 5000 U s.c. and TORADOL did not show any pharmacodynamic effects of the

TORADOL® IM (ketorolac tromethamine)

combination on template bleeding time or kaolin cephalin clotting time. There is no evidence that TORADOL induces or inhibits the hepatic enzymes capable of metabolizing itself or other drugs. Some NSAIDs inhibit renal lithium clearance, leading to increased plasma concentration. This has not been studied with TORADOL. Some NSAIDs reduce the clearance of methotrexate, enhancing its toxicity. This has not been studied with TORADOL. TORADOL has been administered concurrently with morphine in clinical trials without adverse interactions. *Carcinogenesis, Mutagenesis, and Impairment of Fertility:* Studies in mice and rats at oral doses equal to or 2.5 times the parenteral MRHD (Maximum Recommended Human Dose), respectively, showed no evidence of tumorigenicity. Ketorolac was not mutagenic in tests with S. typhimurium, S. cerevisiae, or E. coli and did not cause chromosome breakage in the *in vivo* mouse micronucleus assay. Impairment of fertility did not occur in male or female rats at oral doses 4.5 and 8 times the parenteral MRHD, respectively. *Pregnancy:* Pregnancy Category B: Reproduction studies in rabbits and rats with daily oral doses 1.8 and 5 times the parenteral MRHD, respectively, did not reveal evidence of harm to the fetus. Ketorolac caused delayed parturition and dystocia in rats at oral doses higher than the parenteral MFHD, like other NSAIDs. There are no adequate and well-controlled studies in pregnant women. TORADOL should be used during pregnancy only if clearly needed and no known safer alternatives are available. *Labor and Delivery:* TORADOL is not recommended for use during labor and delivery. *Lactation and Nursing:* After a single oral dose of TORADOL 10 mg to humans, the maximum milk concentration was 7.3 ng/mL and the maximum milk-to-plasma ratio was 0.037. After one day of dosing (qid), the corresponding values were 7.9 ng/mL and 0.025. Use caution when TORADOL is administered to a nursing woman. *Pediatric Use:* TORADOL is not recommended for use in children. *Use in the Elderly:* Because ketorolac is cleared more slowly by the elderly, who are also more sensitive to renal effects of NSAIDs, use extra caution and reduced dosages when treating the elderly. **Adverse Reactions:** Adverse reaction rates from short-term use of NSAIDs are generally 1/2 to 1/10 the rates associated with chronic usage. This is also true for TORADOL. In studies of patients treated for up to 1 year, the incidence of serious and nonserious ADRs, including GI tract ulceration and bleeding (yearly rate 1.2 to 5.4%), associated with oral ketorolac 10 mg, 1 to 4 times per day prn, was comparable to treatment with aspirin 650 mg prn. Be alert for the usual complications of NSAID treatment. The adverse reactions listed below were reported to be probably related to TORADOL in trials in which patients received up to 20 doses of TORADOL 30 mg IM in 5 days. *Incidence greater than 1%:* Body as a whole: edema. GI: nausea,* dyspepsia,* GI pain,* diarrhea. Nervous system: drowsiness,* dizziness, headache, sweating. Injection site pain was reported by 2% of patients in multidose studies (vs 5% for morphine). *Incidence of reported reaction 3%–9%. Reactions occurring in less than 3% are unmarked. *Incidence 1% or less:* Body as a whole: asthenia, myalgia. Cardiovascular: vasodilation, pallor. Dermatologic: pruritus, urticaria. GI: constipation, flatulence, GI fullness, liver function abnormalities, melena, peptic ulcer, rectal bleeding, stomatitis, vomiting. Hemic and lymphatic: purpura. Nervous system: dry mouth, nervousness, paresthesia, abnormal thinking, depression, euphoria, excessive thirst, inability to concentrate, insomnia, stimulation, vertigo. Respiratory: dyspnea, asthma. Special senses: abnormal taste, abnormal vision. Urogenital: increased urinary frequency, oliguria. **Drug Abuse and Physical Dependence:** TORADOL is not a narcotic agonist or antagonist. Subjects did not show any symptoms or signs of withdrawal upon abrupt discontinuation of IV or IM dosing. Patients receiving TORADOL orally for ≥ 6 months have not developed tolerance and there is no pharmacologic basis to expect addiction. TORADOL did not exhibit activity in classical animal studies which are reasonable predictors of opiate analgesic action. *In vitro,* TORADOL does not bind to opiate receptors. Thus, TORADOL does not have central opiate-like activity. **Overdosage:** Lack of experience with acute overdosage precludes characterization of sequelae and assessment of antidotal efficacy. At single oral doses > 100 mg/kg in rats, mice and monkeys, symptoms such as decreased activity, diarrhea, pallor, labored breathing, rales, and vomiting were observed. **Dosage and Administration:** TORADOL may be used on a regular schedule or prn, although current recommendations for pain management are to use analgesics on a regular schedule rather than prn based on the return of pain. For the short-term management of pain the recommended initial dose is 30 or 60 mg IM as a loading dose, followed by half of that (15 or 30 mg) every 6 hours as long as needed to control pain. The recommended maximum total daily dose is 150 mg for the first day and 120 mg/day thereafter. The lower end of the dosage range is recommended for patients under 50 kg (110 pounds), for patients over 65 years of age, and for patients with reduced renal function. CAUTION: Federal law prohibits dispensing without prescription.

U.S. Patent No. 4,089,969 and others.

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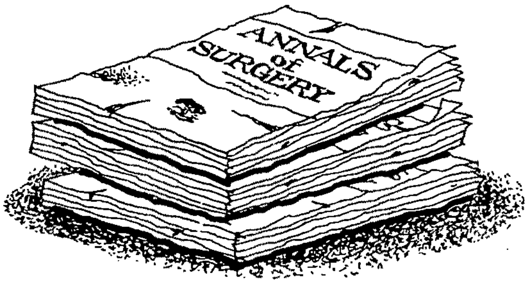

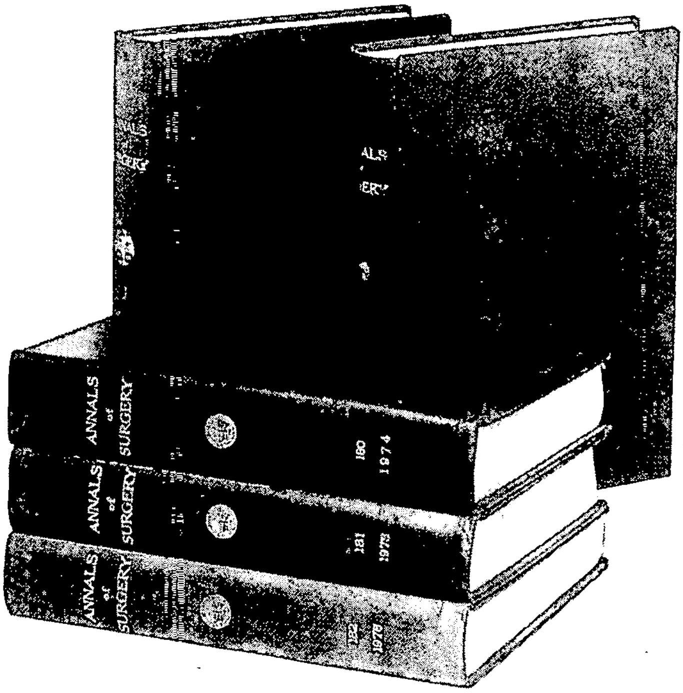

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Management and Long-term Outcome of Aortic Dissection

DONALD D. GLOWER, M.D., RUDIGER H. SPEIER, M.D., WILLIAM D. WHITE, M.P.H.,
L. RICHARD SMITH, M.D., J. SCOTT RANKIN, M.D., and WALTER G. WOLFE, M.D.

From the Department of Surgery, Duke University Medical Center, Durham, North Carolina

All 163 patients admitted to one institution between 1975 and 1988 with aortic dissection were reviewed. Type I and type II patients received grafting of the ascending aorta, with an intraoperative mortality rate of 11%. For type III dissection, management was medical in 53 patients, while 19 required surgery for aortic rupture or expansion, with an intraoperative mortality rate of 11%. The 9- or 10-year survival rates were 29%, 46%, and 29% for types I, II, and III respectively. Of 135 patients with primary aortic dissection, 17 (13%) required subsequent aortic surgery. Cause of late death was other cardiovascular disease in 38%, rupture of another aortic segment in 18%, sudden death in 24%, and other medical conditions in 21%. Although operative therapy for types I and II dissections and reserving operation for selected type III dissections provides acceptable long-term survival, careful follow-up is necessary due to concurrent cardiovascular disease and residual aortic disease.

ALTHOUGH THE LONG-TERM survival rate with aortic dissection has improved since the reports of Hirst[1] and Anagnostopoulos,[2] aortic dissection remains a morbid event, with hospital mortality rates as high as 40%.[3] Furthermore controversy still persists after two decades of debate regarding therapy for descending or DeBakey type III aortic dissection.

Based on many reports suggesting that medical management has survival rates equivalent to or better than surgical therapy for type III dissection,[2,4-11] conventional management for type III dissection has been to reserve operation for those patients with complications of aortic dissection. Recently, however, several authors reported large numbers of patients with type III dissection treated surgically with remarkably low mortality rates.[3,12-18] This has reawakened interest in aggressive surgical therapy and left doubts as to the optimal management therapy for patients with type III dissection.

For types I and II aortic dissection, operative therapy has been the standard of care since the report of Daily.[5] Debate has persisted, however, as to the relative merits of aortic valve replacement *versus* resuspension for aortic valve insufficiency,[3,19] the management of aortic arch involvement with aortic dissection,[20-22] primary aortic repair *versus* aortic grafting,[19,23] and the choice of the inclusion *versus* exclusion techniques of aortic grafting.[24-25] To clarify these issues better, a retrospective study was undertaken of all patients admitted to Duke University Medical Center with the diagnosis of aortic dissection.

Methods

Patient Population

The records of all 163 patients admitted to Duke University Medical Center from 1975 through 1988 with the diagnosis of aortic dissection were examined. Based on the extent of dissection as classified by DeBakey,[3] 68 patients had type I dissection, 23 had type II dissection, and 72 had type III. During the 14-year period examined, most patients were seen by the authors. The diagnosis of aortic dissection was confirmed in all patients by either aortography (135 patients), computed tomography (46 patients), magnetic resonance imaging (8 patients), operative exploration (4 patients), or autopsy (3 patients).

To concentrate on treatment for primary aortic dissection, 28 patients were excluded from further analysis because of previous cardiac or thoracic aortic operation, intraoperative aortic dissection, or death occurring before any therapy was instituted. Nine type I and 1 type II patients presented moribund and expired shortly after admission, either before therapy could be instituted (8 pa-

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Address reprints to Donald D. Glower, M.D., Box 3851, Duke University Medical Center, Durham, NC 27710.
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tients) or before the diagnosis of dissection was apparent (2 patients). Seven type I, five type II, and three type III patients had undergone previous cardiac or thoracic aortic procedures. Nine of these 15 patients with previous operation underwent surgical repair of their dissection with five intraoperative deaths (56%). Four of the six medically managed patients with previous operation were discharged alive, with survival up to 106 months. Two type I and one type II patients developed aortic dissection intraoperatively during other cardiac procedures. Two of these patients died during operation from cardiac failure and one died 18 days after operation from sepsis and complications of cardiac failure.

After discharge patients were followed with serial chest films and with arteriogram, computed tomography, or magnetic resonance imaging as needed to confirm the extent or size of aortic disease. Follow-up was obtained in 131 of 135 patients (97%) and was complete to 1988 in 118 of 135 patients (87%).

Statistical analysis of survival and reoperation outcomes was performed using the Cox–Mantel test to compare Kaplan–Meier curves. Prognostic variables with significant effect on survival were determined from the Cox proportional hazards regression model using both univariate and multivariate analysis. Only those variables significant at the 0.1 level by univariate analysis were examined with multivariate models and stepwise backward elimination of insignificant variables. Because of the small number of patients and deaths in this study, no attempt was made to include all variables of possible prognostic significance in the multivariate analysis. Unless otherwise stated, all results are listed as mean ± standard error of the mean.

Management Technique

Patients with significant hypertension were admitted to the intensive care unit for monitoring and for blood pressure control with beta blockers and vasodilators. All patients managed medically or surgically received long-term treatment with beta blockers and additional antihypertensive agents as needed for blood pressure control.

Surgical candidates with ascending aortic dissection underwent median sternotomy. Patients were cooled to 28 C on cardiopulmonary bypass and the heart was arrested with cold potassium cardioplegia. The ascending aorta was opened longitudinally after clamping the aorta just below the innominate artery, and aortic intima and adventitia were reattached with sutures.[26] In all but 10 patients, a woven Dacron graft then was sutured proximally just above the coronary ostia, and the distal suture line was completed using 3-0 polypropylene. Eight patients underwent primary aortic repair with obliteration of the false lumen[23] and two patients had an intraluminal prosthesis inserted into the ascending aorta.[27] In four patients with intimal tears of the aortic arch, circulatory arrest was used to extend the distal anastomosis up under the arch. Two patients with known arch tears were managed successfully with grafting of the ascending aorta alone, and one patient with an arch tear died from hemorrhage before grafting could be attempted. In patients with type I or type II dissection, aortic valve resuspension was performed in 22 of 66 patients (33%) for acute dissection involving a previously normal aortic valve. Nine patients (14%) required aortic valve replacement for underlying aortic stenosis (four patients), annuloaortic ectasia (two patients), or chronic dissection (three patients). The two patients with annuloaortic ectasia each required Bentall[28] procedures due to aortic root enlargement.

Since 1975 patients with type III dissection were managed selectively, *i.e.*, surgery was offered only to those of acceptable operative risk and with aortic expansion or complications of aortic dissection.[29] Fifty-one patients were managed medically, while 18 type III patients required surgical intervention. Indications for operation were aortic rupture in eight and aortic expansion in 10.

Patients with type III dissection were explored by posterolateral thoracotomy through the left fourth or fifth intercostal space. The distal circulation was supported with left femoral vein to left femoral artery cardiopulmonary bypass in eight patients (44%). A heparinized Gott shunt from the proximal aorta or left ventricular apex to the left femoral artery was used in seven patients (39%). Three patients (17%) were managed without additional perfusion of the distal circulation, due to either life-threatening hemorrhage or inadequate access to the distal circulation. The aorta was opened longitudinally into the true lumen and the tear was identified. A straight woven Dacron graft was secured within the aortic lumen with proximal and distal anastomoses of 3-0 polypropylene, incorporating a strip of Teflon felt wrapped externally around the native aorta. When possible the aortic wall was closed over the graft.

Patient Presentation

The mean age for patients with ascending dissection (types I or II) was 56 ± 12 (standard deviation [SD]) years, with 74% of patients being male. Presentation was acute (less than 2 weeks of symptoms) in 90% of type I patients, as opposed to 38% of type II patients ($p < 0.001$) (Table 1). Type I patients were also more likely to present with rupture (32% *versus* 0%, $p < 0.05$) or tamponade (25% *versus* 0%, $p = 0.05$) than type II patients (Table 2). Aortic insufficiency was clinically evident in 30% of ascending aortic dissections, and 13% of patients with ascending dissection presented in shock. Type I dissection was associated with pulse loss in 48% of patients and dissection

TABLE 1. *Patient Characteristics in Types I, II, or III Aortic Dissection with Medical or Surgical Therapy*

			Type III	
Characteristic	Type I	Type II	Medical	Surgical
Number	50	16	51	18
Age	56 ± 2	55 ± 2	67 ± 1	62 ± 2
Male	39 (78%)	10 (63%)	36 (71%)	12 (67%)
Acute	45 (90%)	6 (38%)*	37 (73%)	9 (50%)
Thoracoabdominal	30 (60%)	—	28 (55%)	11 (61%)
No. medical illnesses	0.6 ± .1	1.0 ± .1*	1.4 ± 0.2	0.5 ± 0.3†

* $p < 0.05$ *vs.* type I.
† $p < 0.05$ *vs.* medical.

below the diaphragm in 60% of patients. Type II patients were more likely than type I to have cardiac disease (75% *versus* 24%, $p < 0.001$) with 56% of type II patients having previous aortic valve disease. Marfan's syndrome also was slightly more common in type II dissection, occurring in 4% of type I and 13% of type II dissections. Renal or visceral ischemia, stroke, and myocardial infarction were relatively uncommon, with myocardial infarction in 2% of ascending dissections and stroke or renal or visceral ischemia each occurring in 6% of ascending dissections.

In 69 patients dissection originated in the descending thoracic aorta. Of these 69 type III patients, 10 patients had dissection extending proximally into the aortic arch, while 59 dissections were strictly distal to the left subclavian artery. Thirty type III dissections were limited to the thoracic aorta, while 39 extended into the abdominal aorta. Age ranged from 47 to 83 years, with a mean of 66 ± 9 (SD) years, which is somewhat older than types I and II dissections ($p < 0.05$) (Fig. 1). There were 48 men and 21 women, with 46 type III patients presenting acutely, while 23 patients had chronic dissections. Compared to medical patients, surgical type III patients were younger, had a higher incidence of rupture on admission, and had fewer major medical illnesses. The number of major medical illnesses was computed as a count of the following organ systems involved with significant disease: cardiac, pulmonary, renal, cerebrovascular, peripheral vascular, and gastrointestinal/biliary. Medical and surgical patients did not differ significantly in sex, distal extent of dissection, or chronicity (Tables 1 and 2).

TABLE 2. *Presenting Complications in Types I, II, or III Aortic Dissection with Medical or Surgical Therapy*

			Type III	
Complication	Type I	Type II	Medical	Surgical
Number	40	12	51	18
Rupture	14 (35%)	0*	2 (4%)	8 (44%)†
Tamponade	10 (25%)	0	0	0
Shock	7 (18%)	1 (8%)	2 (4%)	0
Aortic insufficiency	13 (32%)	3 (25%)	0	0
Pulse loss	19 (48%)	0*	2 (4%)	0
Renal or visc ischemia	3 (8%)	0	2 (4%)	1 (6%)
Stroke	2 (5%)	1 (8%)	0	0
Myocardial infarction	0	1 (8%)	2 (4%)	0

* $p < 0.05$ *vs.* type I.
† $p < 0.05$ *vs.* medical.

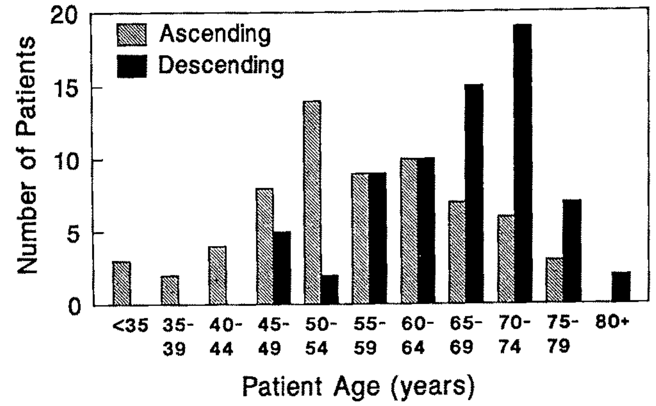


FIG. 1. Age distribution of patients with ascending (type I or II) or descending (type III) aortic dissection.

Results

Types I and II Aortic Dissection

The intraoperative mortality rate was 11% (5 of 47 patients) for type I patients and 14% (2 of 14 patients) for type II patients. The 30-day mortality rates were 26% (12 of 47 patients) and 14% (2 of 14 patients for types I and II dissections, respectively, with 5-year survival rates of 56% ± 9% and 87% ± 8% (Fig. 2). The cause of death with types I or II dissection was cardiovascular disease in 38%, intraoperative in 27%, rupture in 12%, sudden or unexplained in 8%, and other unrelated caused in 15% (Fig. 3).

Subsequent to their original operation, nine patients (17%) with types I or II dissection underwent further surgical procedures for aortic dissection (Table 3). Freedom from dissection-related death or complication requiring operation was 55% ± 8% at 5 years and 39% ± 11% at 9 years for patients with type I dissection and 80% ± 6% and 46% ± 21% for patients with type II dissection at 5 and 10 years, respectively (Fig. 2). Despite the significant incidence of late dissection-related death or reoperation, freedom from late thoracic aortic operation was 87% ± 5% and 60% ± 15% at 5 and 10 years, respectively, for types I or II aortic dissection (Fig. 4). Long-term results for 17 patients receiving aortic valve resuspension were good, with no patient requiring a subsequent aortic valve procedure. Patients with types I or II dissections undergoing aortic valve replacement or no aortic valve procedure had a 30% probability of needing subsequent aortic valve replacement at 10 years (Fig. 5). Of eight patients undergoing

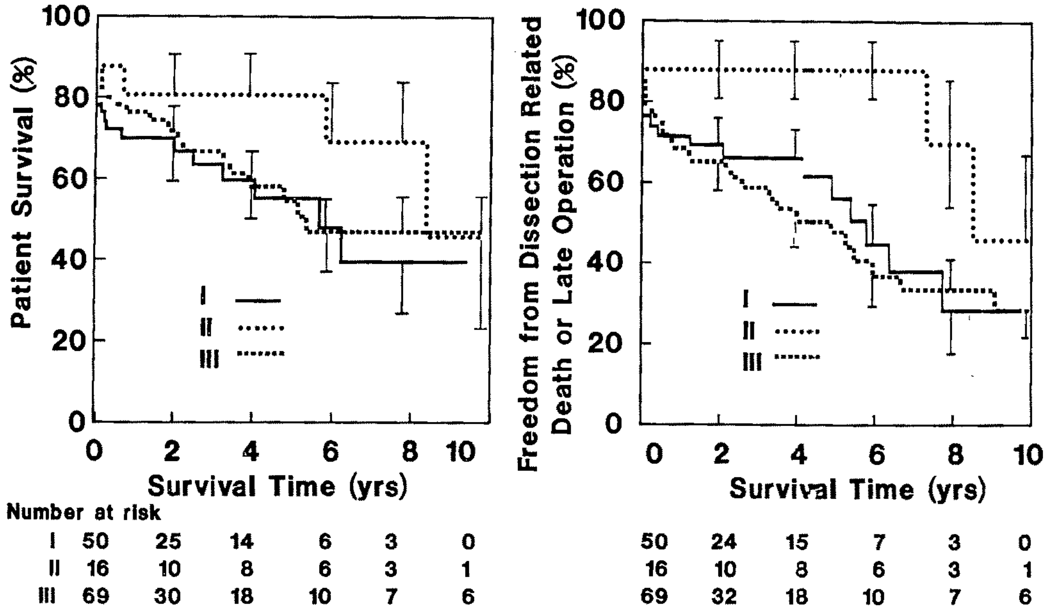


FIG. 2. Patient survival (left panel) and freedom from dissection-related death or late operation (right panel) for type I, type II, and type III aortic dissections.

primary repair of aortic dissection without grafting, two subsequently required Bentall procedures for aortic insufficiency and ascending aortic aneurysm at 8 and 71 months after operation.

For patients with types I or II dissection, only thoracoabdominal dissection (extension of the dissection below the diaphragm) or the presence of a tear in the aortic arch were significantly related to long-term mortality (Table 4, Fig. 6). In this population of 66 patients with types I or II dissection treated surgically, patient age, aortic rupture, complications of dissection at presentation (defined as shock, acute renal failure, visceral ischemia, pulse loss, acute myocardial infarction, or acute stroke), gender, acute presentation, admission date, emergent operation, and the number of medical illnesses all failed to reach significance as independent predictors of death. The 6-month mortality rate did, however, increase from 10%–20% to 40%–60% as age exceeded 60 years (Fig. 7).

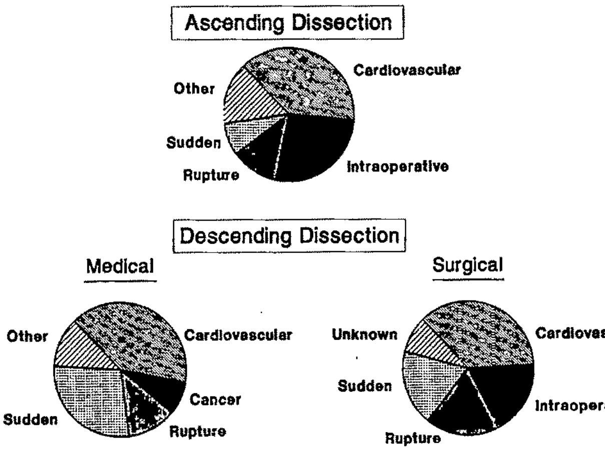


FIG. 3. Cause of death for patients with ascending dissection (top panel) or descending dissection with medical or surgical therapy (bottom panel).

An additional predictor of 30-day mortality rate in patients with acute types I or II dissection appeared to be the experience of the operating surgeon. For acute types I or II dissections during the time period examined, three surgeons each performed nine or more operations for aortic dissection, with a 30-day operative mortality rate of 18% (7 of 39 patients). This mortality rate was significantly better than the pooled 30-day mortality rate of 72% (13 of 18 patients; p = 0.0001) for the seven remaining surgeons who each repaired seven or fewer dissections during the 14-year period examined.

Type III Aortic Dissection

With type III dissection, hospital stay was 14 ± 2 days for surgical patients and 9 ± 1 days for medical patients ($p < 0.05$). The intraoperative mortality rate was 25% (two of eight patients) in patients presenting with rupture, with death resulting from hemorrhage in each patient. No intraoperative deaths occurred when operation was performed electively for aortic expansion. The 30-day hospital mortality rate was 62% (five of eight patients) in patients undergoing operation for rupture, with the three additional hospital deaths resulting from cardiac failure, myocardial infarction, and cardiac arrhythmias in one patient each. All patients repaired surgically for aortic expansion were discharged alive, and no patient developed paraplegia. The 30-day mortality rate was 18% (9 of 51 patients) for patients treated medically, with the cause of death being rupture in two, myocardial infarction in two, and heart failure, renal failure, arrhythmia, sudden death, and hemorrhage at delayed operation in one patient each

TABLE 3. *Subsequent Operations for Complications of Aortic Dissection in Patients Managed Surgically*

Type	Initial Operation	Reoperation	Interval to Reoperation	Reoperation Indication
I	AscAo, Resus	AscAo	69 months	Recurrent dissection
I	AscAo, AVR	AscAo	38 months	False aneurysm
I	AscAo	AscAo	3 months	Mediastinitis, anast dehiscence
II	Primary	AVR, AscAo	8 months	Aortic insuff
II	AscAo, AVR	AVR, AscAo	102 months	Aortic insuff
II	Primary	Bentall	71 months	Aortic insuff, aortic expansion
I	Bentall	DescAo, Arch, ThorabdAo	30 months 55 months 91 months	Distal dissection, Marfan's syndrome
I	AscAo, Resus	DescAo, AxFem	3 months	Distal ischemia
I	AscAo	FemFem	1 day	Distal ischemia
III	DescAo	DescAo	65 months	Distal dissection
III	DescAo	DescAo	2 months	False aneurysm

Arch = arch graft, AscAo = ascending aortic graft, AVR = aortic valve replacement, DescAo = descending aortic graft, Primary = ascending aortic primary repair, Resus = aortic valve resuspension.

(Fig. 3). One medical patient dying of rupture was moribund at admission, and a second medical patient died of rupture acutely after an otherwise stable course. For all 69 type III patients, 5- and 10-year survival rates were 48% ± 7% and 29% ± 8%, respectively (Fig. 2). Late aortic rupture was confirmed in one medical patient after 58 months and in two surgical patients after 5 and 47 months.

Eight patients (12%) with type III aortic dissection underwent aortic operations subsequent to initial therapy. Two medical patients required grafting of the descending aorta for rupture or expansion after 9 days and 17 months of medical therapy, respectively. Three medical patients underwent grafting of abdominal aortic aneurysms after 2, 6, and 43 months. One medical patient required grafting of the ascending aorta after 22 months for ascending aortic aneurysm. Two surgical patients required reoperation 2 and 65 months after operation, one for false aneurysm of the proximal anastomosis and one for progression of descending thoracic dissection (Table 3). For patients with type III aortic dissection, freedom from dissection-related death or complication requiring operation were 54% ± 8% at 5 years and 46% ± 8% at 10 years (Fig. 2). Freedom from late thoracic aortic operation was 92% ± 10% and 73% ± 22% at 5 and 10 years, respectively (Fig. 4)

By univariate analysis for type III dissection, the only factors significantly associated with impaired long-term survival were the presence of complications of dissection at presentation, increasing patient age, aortic rupture at presentation, admission date, and acute presentation (Table 5). By multivariate analysis, only the presence of complications of dissection on admission, increasing patient

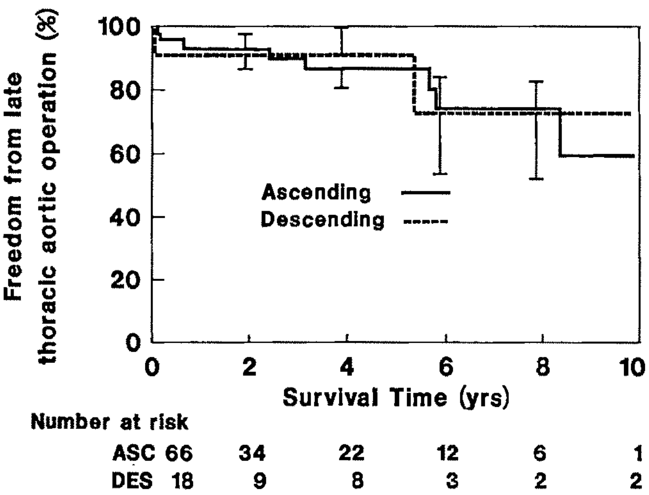


FIG. 4. Freedom from thoracic aortic reoperation after grafting of the ascending or descending aorta for aortic dissection.

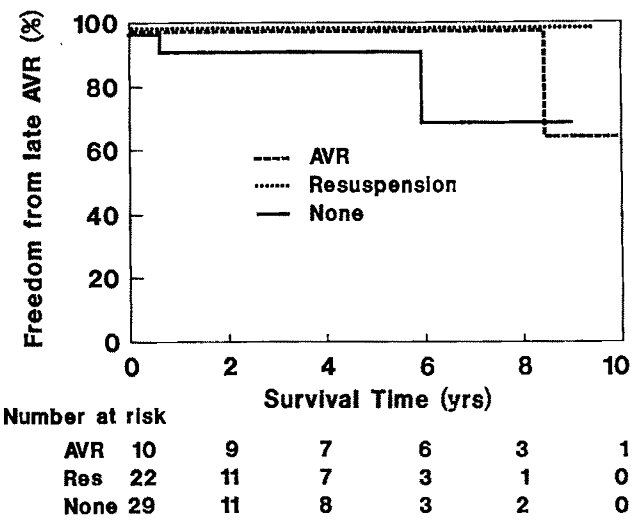


FIG. 5. Freedom subsequent aortic valve replacement in patients with ascending dissection receiving initial aortic valve replacement (AVR), resuspension, or no aortic valve procedure.

TABLE 4. *Univariate and Multivariate Analysis of Variables Significantly (Univariate p < 0.1) Predictive of Death for Ascending Aortic Dissection*

	Univariate		Multivariate	
Variable	Chi^2	p	Chi^2	p
Arch tear	3.94	0.047	2.54	0.11
Thoracoabdominal dissection	3.60	0.058	2.82	0.09

age, and aortic rupture were independent predictors of death (Figs. 7 and 8). Among the complications of dissection present at admission, shock, acute renal failure, visceral ischemia, acute myocardial infarction, and pulse loss were each associated with no survival more than 5 months in type III dissections. Six-month mortality rate increased dramatically from less than 20% to 60% as age approached 70 years in descending dissections (Fig. 7). Proximal or distal extent of dissection, gender, the presence of an arch tear, and concurrent medical illness all failed to reach significance as independent predictors of survival in patients with type III aortic dissection. Of particular importance in type III dissections, no significant difference in survival was noted between patients treated medically and those treated surgically ($p = 0.5$) (Fig. 9). Estimates of maximal aortic size by computed tomography or aortography were significantly related to subsequent risk of rupture or need for operation (Fig. 10). Dissections larger than 5.5 cm to 6.0 cm in diameter had greater than a 50% probability of rupture or operation.

Discussion

The earliest descriptions of aortic dissection were those by Morgagni in 1761[30] and by Nicholls in his autopsy of King George II in 1760.[31] Clear distinction between dissections involving different portions of the aorta was not made until 1955, when DeBakey described nine different subgroups of dissections along with the first large series of surgical results.[32] In 1964 and again in 1982, DeBakey revised the classification of aortic dissection into three types based on the location of the aortic tear[33] or the extent of aortic involvement.[3] In 1970 Daily et al.[5] noted that descending dissections generally had better survival with medical therapy than with surgical therapy, unless complications of dissection or increase in aortic size mandated surgical intervention. Based on these observations, the Hume,[34] Stanford,[5] Reul,[12] Najafi,[35] Alabama,[7] Inoue,[36] and MGH[10] classifications have all distinguished dissections involving the ascending aorta from those that do not.

Since the reports of Daily[5] and others, standard treatment of descending or type III dissection has reserved surgical therapy for patients with complications or aortic enlargement. Yet, despite many published studies of type III aortic dissection, debate as to the optimal therapy for type III dissection has persisted due to lack of controlled data and due to improved results with surgical therapy. Systematic analysis of prognostic variables in type III dissection has been reported from only a small number of institutions.[18,37,38] Similarly data including 10-year follow-up and series of at least 150 patients are scanty, coming from four institutions: Baylor University,[3,18] Stanford University,[13,37] the Mayo Clinic,[17,19] and the Massachusetts General Hospital.[10]

In the present series, surgical management for selected high-risk type III patients produced results similar to those of other recent surgical series. The 30-day mortality rate of 28% for surgical type III patients correlates with that

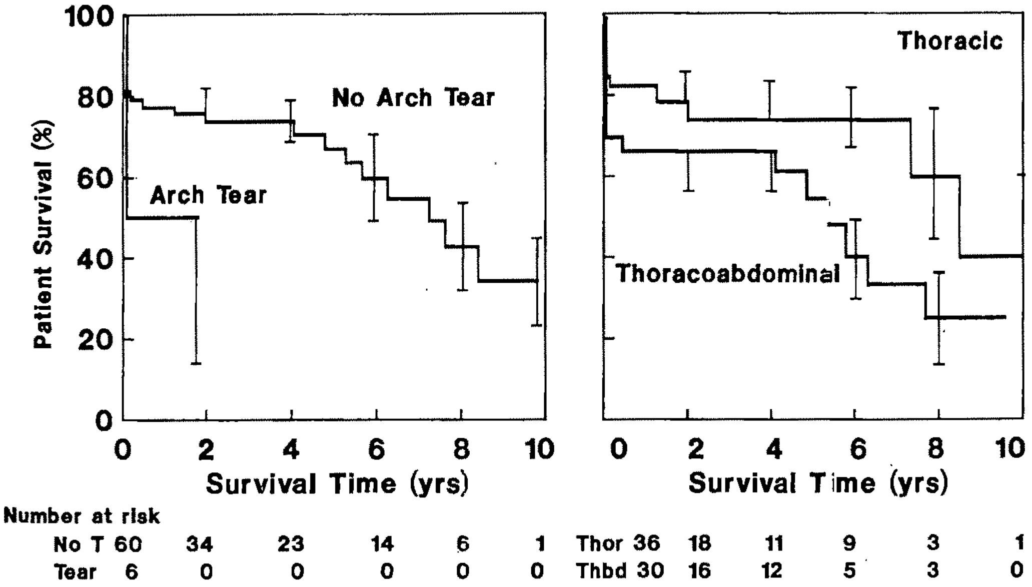


FIG. 6. Survival with or without an arch tear (left panel) and with thoracic or thoracoabdominal dissection (right panel) in patients with ascending aortic dissection.

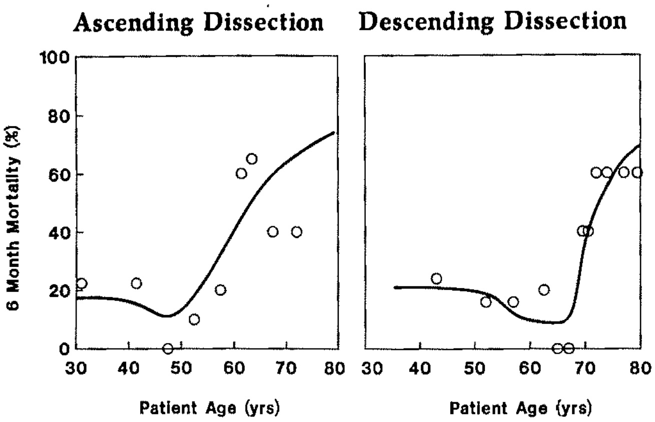


FIG. 7. Influence of patient age on 6-month mortality rates in patients with ascending or descending aortic dissection.

TABLE 5. *Univariate and Multivariate Analysis of Variables Significantly ($p < 0.1$) Predictive of Death for Descending Aortic Dissection*

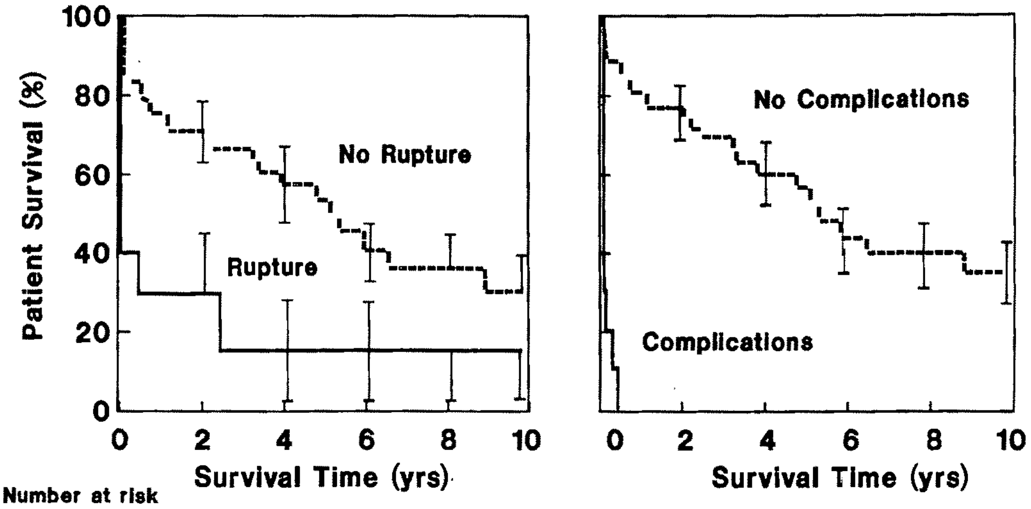
	Univariate		Multivariate	
Variable	Chi^2	p	Chi^2	p
Presenting complication of dissection	57.80	0.0001	25.76	0.0001
Age	12.98	0.0003	11.96	0.0005
Rupture	7.43	0.006	6.24	0.01
Recent admission data	6.73	0.01	2.09	0.15
Acute presentation	3.87	0.049	1.91	0.17

of other series (Table 6), and the long-term survival rate shown in Figure 2 also parallels that reported by Crawford,[18] Rizzoli,[39] and Sutton.[8] Differing patient selection criteria between series may account for some of the differences in mortality rates. The increased likelihood of rupture or operation with aortic diameter greater than 5.5 cm to 6 cm in type III dissection (Fig. 10) compared well with the 5.5-cm criterion for operation proposed by Crawford et al.[18]

Data from the current study (Fig. 9) imply that selective management of type III dissection, as practiced at this institution, can provide acceptable results with comparable survival between medical and surgical groups. Figure 9 does not, however, imply that surgical and medical treatment were equivalent because all surgical patients had aortic rupture or aortic expansion and should have had worse survival if surgical intervention had been ineffective. For patients with uncomplicated descending aortic dissection, a retrospective, multivariate analysis of patients from two institutions similarly found no difference in survival or incidence of late operation between medical and surgical therapy.[38]

Type I dissection is known to be associated with a particularly poor prognosis without surgical intervention.[5] The number of type I patients arriving at this institution sufficiently ill to die before therapy could be instituted emphasizes the urgency with which acute type I dissection must be managed. Other than the current report, very few surgical series have provided data on the incidence and outcome in patients not receiving operation for type I or II dissection due to death occurring before operation or due to other severe medical disorders.[18]

Intraoperative aortic dissection was rare at this institution but was highly morbid with all three patients dying of cardiac failure, perhaps due to severity of underlying cardiac disease and origin of the dissection in the ascending aorta. Murphy et al.[40] reported a 40% (6 of 15 patients) mortality rate with intraoperative dissection, while Carey[41] reported survival in six of seven patients in whom intraoperative dissection originated at the femoral artery can-



Number at risk					
No R 59	29	17	10	6	5
Rupt 10	3	1	1	1	1
No 59	32	18	10	7	6
Cx 10	0	0	0	0	0

FIG. 8. Survival with or without rupture (left panel) and with or without other presenting complications of dissection (right panel) in patients with descending aortic dissection.

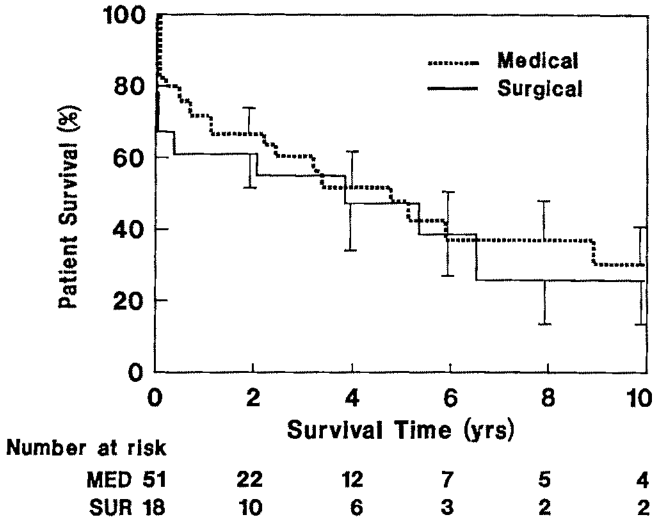


FIG. 9. Patient survival with medical or surgical therapy in descending aortic dissection.

nulation site. The high mortality rate of intraoperative dissection in the current series and that of Murphy may reflect the more serious nature of dissection originating in the ascending aorta as opposed to the femoral artery.[41]

In the present series, the mortality rate was also significant when patients with previous cardiac or thoracic aortic procedures required acute surgical intervention. Similarly Murphy reported only two of nine survivors with operation for acute ascending aortic dissection occurring after a previous cardiac surgical procedure, and these two survivors had the dissection repaired within hours of the original cardiac procedure.[40] Early diagnosis and operative intervention therefore would appear to be important in managing acute types I or II dissection after other cardiac procedures. On the other hand, patients presenting with chronic ascending aortic dissection after previous cardiac operations may fare better, with five of six such patients reported by Orszulak[42] surviving repair of aortic dissection.

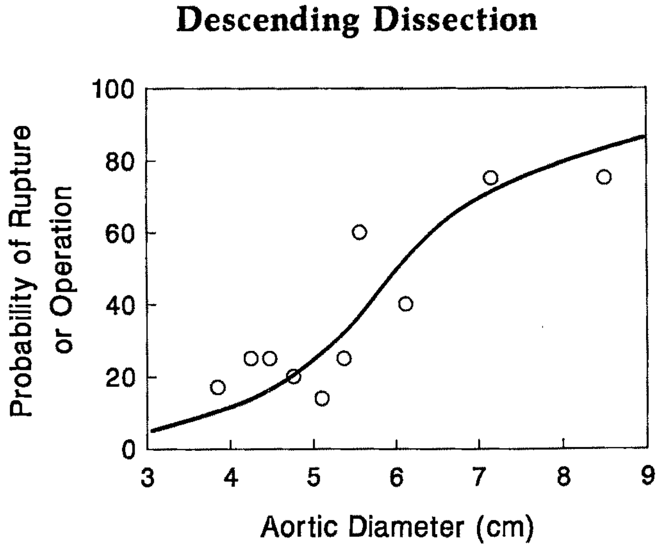


FIG. 10. Effect of maximal aortic diameter on probability of aortic rupture or aortic operation in descending aortic dissection.

TABLE 5. *Medical and Surgical Deaths (*30-day, Hospital, or Operative) from Larger Studies of Type III Aortic Dissection*

Institution	Author	Medical	Surgical
Baylor	Crawford 1988		41/317
Lyon, France	Kirkorian 1988	15/42	
Mayo	Jex 1986		18/64*
MGH/Yale	Cambria 1989	13/90	27/58
Milan, Italy	Ruberti 1988		11/29*
Stanford	Miller 1984		15/54*
Texas Heart Institute	Reul 1975		19/91*
U Alabama	Appelbaum 1976	7/27	8/21
U British Columbia	Fradet 1988	4/27	6/20
U Virginia	Mills 1979	10/17	5/14
Uppsala, Sweden	Bergholm 1984	5/22	12/13
Zurich, Switzerland	Von Segesser 1988		8/31
Total		53/225 (24%)	170/712 (24%)

Increased deaths in types I or II dissection with aortic involvement below the diaphragm (Fig. 6, Table 4) has been reported by Cambria[43] and DeBakey.[3] These additional deaths may have resulted from increased risk of distal organ ischemia and distal aortic rupture. Indeed the occurrence of peripheral pulse loss or visceral ischemia as presenting complications of descending dissection was associated with a significant mortality rate in the current study (Table 5). Crawford et al.[18] have reported large numbers of patients in whom both the thoracic and the abdominal aorta were grafted. The morbidity of extensive aortic grafting still leaves doubt as to the role for such procedures.

The association between native aortic valve disease and dissection, including chronic type II dissections, was noted by McKusick in 1957.[44] Type II dissection may be more likely when previous aortic disease is limited to the ascending aorta (as with ascending aortic aneurysm associated with aortic valve disease), while type I dissection may more often result from chronic hypertension and diffuse aortic disease.

Spencer and Blake[45] first described successful resuspension of the aortic valve for regurgitation produced by aortic dissection in 1962. While several authors have favored aortic valve replacement for aortic valve insufficiency due to dissection,[3,46-48] many institutions have reported good short-term results for aortic valve resuspension.[7,19,25,49-52] Long-term results after aortic valve resuspension have been limited to the report of Miller et al.[13] with two reoperations in 134 patient-years of follow-up[49] and the report of Jex et al.[19] with 1 of 18 patients ultimately requiring reoperation. Data from the current study (Fig. 5) demonstrate that the failure rate of aortic valve resuspension with up to 9 years of follow-up can be low in patients with acute dissection, a previously normal valve, and absence of annuloaortic ectasia. Previous dissatisfaction with aortic valve resuspension may have resulted from suboptimal patient selection.

Primary repair of aortic dissection was described by DeBakey in 1955 for type III dissections[32] and in 1964 for ascending aortic dissection.[33] Olinger[23] recently reported a series of 14 patients undergoing primary repair of ascending aortic dissection, with one patient requiring subsequent aortic valve replacement and repair of a sinus of valsalva aneurysm. In the present series, however, a significant 25% incidence of late ascending aortic aneurysm and aortic insufficiency requiring operative correction was noted. Three of five patients with primary aortorrhaphy reported by Appelbaum et al.[7] died from redissection. Jex et al.[19] also noted recurrent aortic dissection in 4 of 13 patients treated with primary aortorrhaphy as opposed to 3 of 59 patients treated with an aortic graft ($p = 0.005$). Although the patient numbers are small, these results suggest that interposition grafting of ascending aortic dissection should be the procedure of choice for repair of aortic dissection.

Just as the merits of primary aortic repair have been debated, the choice of the inclusion[26] *versus* exclusion[13] of the native aortic wall around the aortic graft has been controversial. Massimo et al.[53] reported that the incidence of late death due to persistent distal dissection was higher with the inclusion technique than with aortic exclusion. Similarly Kouchoukos et al.[24] reported a higher incidence of late reoperation on the ascending aorta or aortic valve using the inclusion *versus* the exclusion technique (23% ± 5% *versus* 10% ± 7% at 5 years). Borst,[25] on the other hand, argued that the inclusion technique should better obliterate the false channel and decrease secondary rupture. Data from the current study indicate that the inclusion technique can provide a low incidence of reoperation at 5 years (13% ± 6%) (Fig. 10) quite comparable to the 13% ± 4% incidence of dissection-related reoperation at 5 years reported by Haverich et al.[37] with the exclusion technique. Although the reoperation rate at 10 years was somewhat higher in the current study with the inclusion technique (40% ± 15%) than that reported by Haverich et al.[37] with aortic exclusion (23% ± 6%), these results do not differ significantly due to small patient numbers.

The greatest source of death in the current study was associated cardiovascular disease (Fig. 3). The fact that known pre-existing cardiac, renal, or cerebrovascular disease were not of prognostic significance suggests that cardiovascular disease was more pervasive in this patient population than was clinically evident. The significant long-term mortality rate and the prominence of cardiovascular, sudden, and rupture-related death suggests that careful follow-up is needed, both for cardiovascular disease and for complications of aortic dissection. The significance of residual aortic disease also was demonstrated by Heinemann et al.,[54] who found that 17% of 86 patients with acute ascending aortic dissection developed significant dilation of the distal aorta after repair of the ascending aorta. These results parallel those observed for all patients with Marfan's syndrome, with which 48% of deaths are due to aortic dissection or rupture and 36% are due to sudden death or other cardiovascular disease.[55]

The influence of the surgeon's experience on operative mortality rates in aortic dissection has not been documented previously. Several factors that might contribute to the association between the surgeon's experience and operative mortality rate include the relative infrequency of aortic dissection in most thoracic surgical practices, the acuity with which most patients present, the technical demands of operations for aortic dissection, especially when exposure is difficult and tissues are suboptimal, and the judgment required in technical aspects such as the handling of distal perfusion during aortic clamping. The implication is that, even in a large medical center, mortality rates might be improved through management of aortic dissection by a few individuals.

One potential weakness of this study was the number of patients dying suddenly, especially in the type III group, without being able to distinguish sudden death by aortic rupture from death by other causes, such as stroke or myocardial infarction. These sudden deaths generally occurred at home or in outlying hospitals and again emphasize the need for long-term follow-up, both for associated cardiovascular disease and for aortic disease.

Remaining issues include whether surgical therapy might benefit type III patients other than those with rupture or expansion. The low surgical mortality rate in the group operated on electively for expansion suggests that a relatively low operative mortality rate could be achieved under elective conditions for other patients. Patients with acute dissection and those with retrograde dissection potentially could benefit more frequently from operation more than currently thought. Although retrograde aortic dissection is not uncommon, few data are available regarding outcome of aortic dissections originating in the descending aorta but extending retrograde into the aortic arch or ascending aorta. Pinet[56] found 4 of 32 patients (13%) with dissection originating in the descending aorta to have involvement of the ascending aorta, not unlike the aortic arch involvement seen in 10 of 69 patients (14%) with type III dissection in the present study. Cipriano[57] reported deaths in three of six patients with retrograde extension of dissection into the ascending aorta. The three retrograde patients managed surgically in the current series gave mixed results, with one operative death, one long-term survivor, and one patient dying suddenly at home 1 month after operation. While patients with acute or retrograde type III dissection may be at increased risk for death, extent of this risk and the optimal management of these patients awaits further data.

A related issue is whether those patients with ascending dissection and increased risk due to an intimal tear in the aortic arch might also benefit from a more aggressive approach. Several authors[18,20,21,53,58] have grafted the aortic arch when involved, particularly with an intimal tear in the aortic arch or compromise of brachiocephalic vessels. Nonetheless the 20% to 34% mortality rate of arch replacement has tended to discourage its use. Furthermore Miller et al.[13] were unable to show any significant relationship between resection of the primary intimal tear and subsequent risk of late valvular or aortic operation, although the reoperation rate was lower in patients with tear resection.

Operation remains the therapy of choice in patients with type I or II aortic dissection. Particularly in type I patients in whom presentation is often acute, operation should be performed on an urgent basis because the risk of death from rupture is otherwise significant. Type II patients more often present with chronic dissection and with previous aortic valve disease. Aortic valve resuspension in patients without native aortic valve disease has resulted in excellent freedom from long-term valve failure. The practice of reserving surgical intervention for type III patients with rupture or expansion has produced acceptable outcome at this institution during the last 14 years. The late incidence of aortic complications such as rupture, further dissection, or expansion often requiring surgery was a small but significant contributor to late death. Careful and aggressive follow-up with chest film, computed tomography, or angiography is essential in minimizing late morbidity and mortality rates due to residual aortic disease and concurrent cardiovascular disease in patients with aortic dissection.

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Ten-year Experience with Cyclosporine as Primary Immunosuppression in Recipients of Renal Allografts

NICHOLAS L. TILNEY, M.D.,* AMY CHANG, R.N.,‡ EDGAR L. MILFORD, M.D.,† W. DAVID WHITLEY, M.D.,*
J. MICHAEL LAZARUS, M.D.,† ELEANOR L. RAMOS, M.D.,† TERRY B. STROM, M.D.,†
CHARLES B. CARPENTER, M.D.,† and ROBERT L. KIRKMAN, M.D.*

Cyclosporine has been used as primary immunosuppression in renal allograft recipients in our unit for the past decade. The overall clinical experience and long-term effects of the agent are reviewed. There were 461 consecutive recipients of kidney grafts; 379 received grafts from cadaver donors (CAD) and 82 from living related donors (LRD). Four separate clinical protocols were used sequentially using progressively decreasing doses of CyA; azathioprine was added in group 4 recipients of LRD grafts, and in patients receiving secondary CAD grafts (group 5). The patient mortality rate was less than 5%, with sepsis being the prime contributor. The majority of kidney grafts were lost within the first 2 months after operation; those that never functioned were found almost invariably to have been irreversibly rejected. During the subsequent years of follow-up, attrition of CAD grafts was significantly greater than LRD grafts. In contrast, the attrition rate of primary and secondary CAD grafts was the same after the first 3 months, emphasizing the importance of early immunologic graft destruction. Primary nonfunction occurred in 49% of CAD kidneys and 17% of LRD grafts; however 71% of initially nonfunctioning LRD grafts never functioned at all compared to 34% of CAD grafts, the majority of such organs undergoing fulminate rejection. Individual graft loss after 1 year was almost inevitably due to chronic rejection; there were no differences in long-term allograft function among the treatment groups. Although CyA has improved overall results of kidney transplantation, chronic rejection remains a major unresolved problem.

From the Departments of Surgery, Medicine,† and Nursing,‡*
Brigham and Women's Hospital, Boston, Massachusetts

CYCLOSPORINE (CYA) WAS FIRST used in recipients of renal allografts by Calne in 1977.[1] Despite the necessity of a prolonged learning curve to appreciate fully the nuances and side effects of the agent, it has now virtually replaced azathioprine as primary immunosuppression in the majority of transplant units,[2-5] and has contributed to important improvements in the results of engraftment of several other organs, particularly livers and hearts. Despite its obvious clinical effectiveness, however, the optimal therapeutic regimen for CyA has been difficult to determine and its sequellae not insignificant. In particular, its often profound nephrotoxic effects remain poorly understood and inadequately controlled.[6] Early nephrotoxicity usually can be reversed by reducing the dosage; however the question as to whether long-term maintenance immunosuppression with CyA causes progressive deterioration of renal function over time because of irreversible structural changes within the graft parenchyma remains unanswered. As such concerns still persist even after a decade of use, many groups have administered low doses of CyA in combination with other immunosuppressive modalities, given sequential therapy in which CyA is not initiated until renal function has become established, or converted to azathioprine at various times after engraftment.[7-11] Other groups, however, continue to use CyA in combination with adjunctive steroids.[5]

The renal transplant group at the Brigham and Women's Hospital first treated recipients of cadaver renal allografts with CyA in 1979 with protocols that had evolved from Calne's earlier findings[12]; by 1981, CyA was used preferentially over azathioprine. This report summarizes the overall clinical experience during a 10-year period with CyA as the primary immunosuppressive agent in kidney graft recipients, and emphasizes the long-term effects of the agent.

Materials and Methods

The complete course of each of 461 consecutive CyA-treated recipients of renal allografts transplanted between November 1, 1979 and November 1, 1989 was reviewed.

Address reprint requests to Nicholas L. Tilney, Department of Surgery, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.
Dr. Strom's present address is Beth Israel Hospital, Boston, MA, 02115.

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Follow-up varied between 4 and 98 months. There were 379 patients who received grafts from cadaver donors (CAD) and 82 from haploidentical living related donors (LRD); individuals undergoing a second or third transplant were counted as new patients. Recipients of human lymphocyte antigen (HLA) identical grafts were not treated with CyA and are not included in this study. All recipients of CAD and LRD grafts were given between one and five random blood transfusions before transplantation; donor-specific transfusions were not used in LRD recipients.

Demographics

The general characteristics of the recipient population are noted in Table 1 and have not varied during the years studied. Ten per cent of the patients (n = 46) were type I insulin-dependent juvenile-onset diabetics, the majority of whom (70%) received CAD grafts. All individuals had normal hepatic function before transplantation but four patients, whose liver function tests had been chronically elevated but stable for at least 6 months and did not worsen throughout the follow-up period. Methods of immunologic screening and tissue typing were used as described previously.[13]

Distribution of CAD kidneys was mediated under the aegis of the New England Organ Bank, the policies of which give some precedence to highly sensitized patients. The majority (90%) of grafts were machine perfused and the remainder were stored on ice. The cold ischemic time before implantation ranged between 12 and 60 hours; the mean was 36 hours. Living related donor kidneys were flushed with a cold intracellular solution immediately on removal then implanted directly into the recipient. The time for vascular anastomosis varied between 30 and 45 minutes. All patients received furosemide on revascularization of the graft.

CyA Serum Levels

The radioimmunoassay for CyA was performed using the technique of Donatsch et al.[14] To obtain trough levels, sera were collected 24 hours after the previous daily dose. Serum levels were determined three times each week during the initial hospitalization, once each week in the early postoperative period, and as indicated during follow-up clinic visits. For the first half of the experience, serum levels of about 200 ng/mL were considered optimal; subsequently a range of 100 to 150 ng/ml was retained in the early weeks after operation; levels generally were maintained within 50 to 100 ng/mL after 3 months.

TABLE 1. *Patient Demographics*

Recipients	No.	M	F	Age (mean, years)	Diabetic (%)
LRD	82	62	20	33	14 (17%)
CAD	379	237	142	40	32 (8%)
Total	461	299	162	37	46 (10%)
Number of transplants	1	2	3	4	
CAD	312 (82%)	51 (13%)	13 (3%)	3	
LRD	78 (96%)	2 (2%)	2 (2%)		

LRD, living related donor; CAD, cadaver donor.

Clinical Protocols

The initial loading dose of CyA was lowered progressively during the period reviewed in this study. Four separate protocols were used sequentially in recipients of primary grafts in an effort to reduce drug toxicity and improve outcome; numbers of patients in each group are relatively comparable (Table 2). Group V comprised recipients of second or subsequent (secondary) CAD grafts who received triple therapy.

The drug was initiated intravenously the day of surgery at one third the equivalent oral dose, then administered by mouth after 2 or 3 days. After 2 weeks and then at monthly intervals thereafter, the dose was reduced by decrements of 2 mg/kg until it reached maintenance levels of 5 to 8 mg/kg/day by 3 months, 3 to 5 mg/kg/day by 6 months, and lower thereafter. Adjunctive steroids were used in all patients, with prednisone (30 mg) given daily for the first 3 months, then gradually reduced to about 10 mg/day by 6 months, and ultimately to 2 to 3 mg/day in most patients. The maintenance dose of steroids was never discontinued. In patients receiving triple therapy (groups 4 and 5), the doses of CyA and steroids were also reduced slowly during 3 months if graft function remained satisfactory; the dose of azathioprine remained at 2 mg/kg/day indefinitely unless a lower dose was dictated by leukopenia.

The clinical management of kidney graft recipients in our unit has been based on standard protocols.[12] Needle biopsies were performed under ultrasound guidance in poorly or nonfunctioning allografts as necessary in an attempt to differentiate between acute tubular necrosis (ATN), CyA toxicity, and rejection. Diagnosis of CyA nephrotoxicity was usually one of exclusion because there were few specific findings on biopsy; elevated levels of serum CyA were adjusted and the effects of concomitant administration of other pharmacologic agents that may interact agonistically or antagonistically with CyA were examined.[15] Episodes of acute rejection, histologically proved or diagnosed clinically, were treated with intravenous methyl prednisone (Solumedrol, 1 g/day for 3 days). If an acute episode was not reversed with one or two such pulses, a single 10-day course with the anti–T-cell monoclonal antibody, OKT3 (Ortho Pharmaceuticals,

TABLE 2. *CyA Protocols*

Group	Period Used	Initial Protocol CyA (mg/kg/d)	Azathioprin (mg/kg/d)	Prednisone (mg/d)	No. of Recipients CAD	LRD
I	Nov'79–Oct'83	17	—	30	93	29
II	Nov'83–Oct'85	15	—	30	91	19
III	Nov'85–Oct'87	12	—	30	115	16
IV	Oct'87–Nov'89	8	2	30	110	18
V	Mar'86–Nov'89	8	2	30	30	—

LRD, living related donor; CAD, cadaver donor.

* Group V, recipients of secondary cadaver grafts.

Raritan, NJ), has been used since 1986. If the graft still failed to respond or another major rejection occurred within the first 3 months, further treatment was rarely entertained and the recipient returned to dialysis as necessary. Patients were followed at appropriate intervals in the transplant clinic and/or by their referring nephrologist. If rejection affected allograft function later in the postoperative period (more than 3 months), an oral pulse (100 mg Prednisone/day for 3 days) was given. If chronic rejection or recurrent nephritis was found on late biopsy, further immunosuppressive treatment was rarely administered.

Statistics

Actuarial data were calculated using the Kaplan–Meier survival curve method; the groups were compared by the log rank test. The chronic attrition rate was assessed by the slope of graft loss (t½) after 3 months. Comparison of primary allograft function between LRD and CAD allografts was determined by chi square analysis.

Results

Patient Survival and Complications

The actuarial survival rates of recipients of CAD and LRD kidneys are shown in Figure 1. The overall mortality rate within the first year was 5% for recipients of CAD grafts and 4% for recipients of LRD grafts; no significant differences in mortality rates were noted among those with primary or secondary grafts (Fig. 2). In contrast the attrition rate for recipients with juvenile-onset diabetes mellitus was greater than among nondiabetic patients in the long-term; although their mortality rate at 1 year was comparable, 24% had died within 3 years (*versus* 7% of nondiabetics), usually of atherosclerotic and septic complications of their disease. Thus, at the end of the follow-up period, 9% of all recipients in the series had died. When graft recipients were divided into the various treatment groups 1 to 5 (Table 2) and their mortality rates compared, no statistical differences were noted.

The causes of death are enumerated in Table 3, with sepsis being the major contributor. Pneumonia occurred most commonly, often from opportunistic organisms; of 10 fatal cases developing within the first year of transplantation, *Pneumocystis carinii* was the responsible organism in four instances, *Legionella pestis* in one, and miscellaneous bacteria in five. Of those dying after 1 year, two patients had primary Pneumocystis, while the remainder developed pneumonia as part of a spectrum of liver failure, pancreatitis, or generalized sepsis.

The complication rate was higher among recipients of CAD grafts than those receiving grafts from LRD, with pneumonia and liver disease occurring most frequently. A cluster of 13 cases of *P. carinii* infection developed within a 4-month period in 1984 to 1985, from which there were five fatalities. None of these individuals had received prophylactic trimethoprim-sulfa (Table 3).

Allograft Survival

The actuarial rates of overall survival of allografts from both CAD and LRD sources are shown in Figure 1. Most failed kidneys are lost within the first 2 months after transplantation; 19% and 17%, respectively, never func-

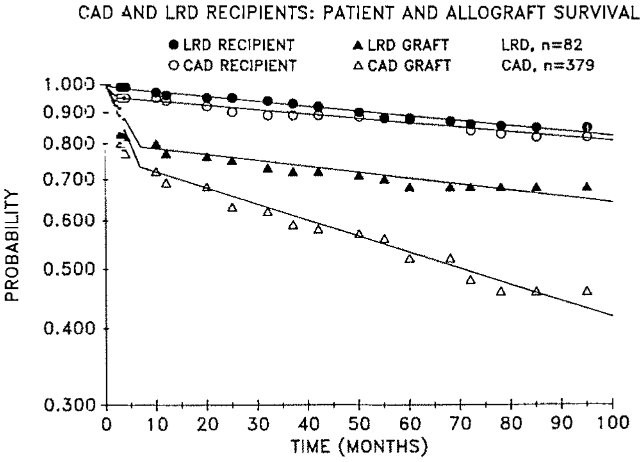


FIG. 1. Actuarial semi-log plot of recipient survival and allograft function. The attrition rate of cadaver grafts (△) over time is significantly ($p < 0.05$) greater than that of grafts from living related sources (▲).

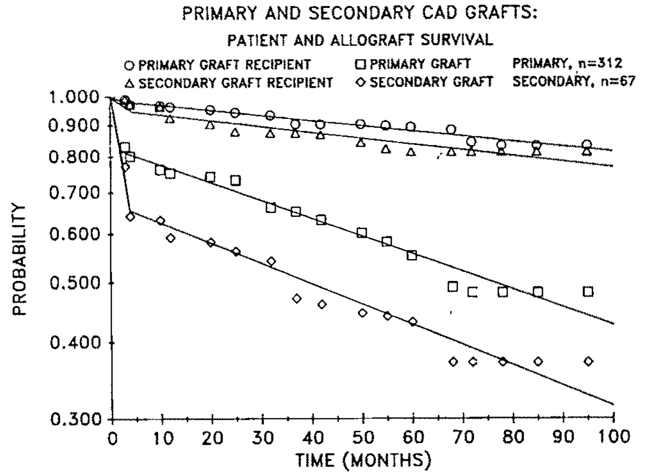
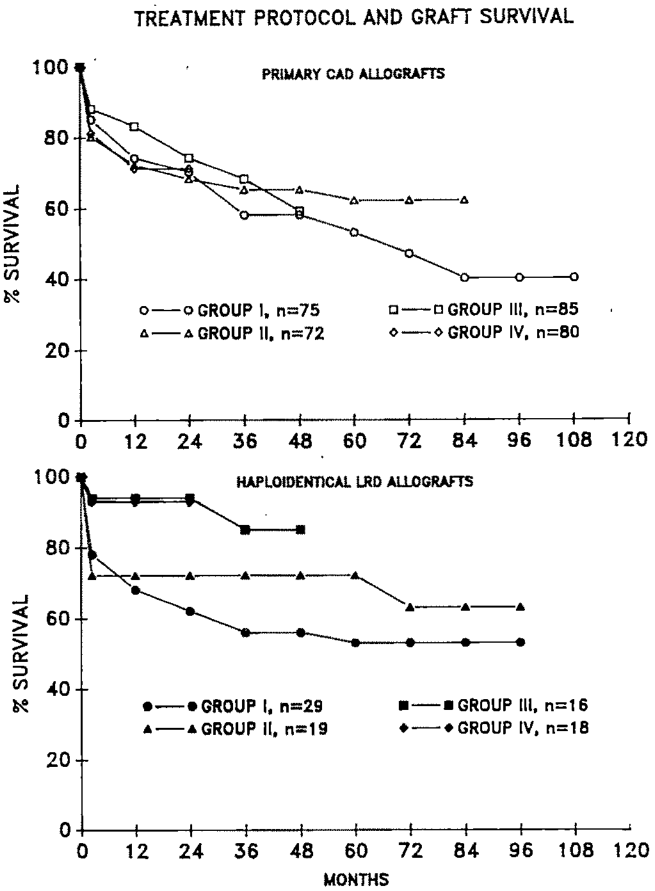


FIG. 2. Actuarial semi-log plot of recipient survival and graft function of primary and secondary cadaver allografts. First cadaver grafts (□) performed significantly ($p < 0.05$) better at all time points than do secondary (◇) grafts.



FIGS. 3A and B. Function of allografts in the various recipient treatment groups are plotted. (A) For primary cadaver grafts, treatment protocols made no significant difference in graft outcomes. (B) In contrast actuarial survival of grafts from haploidentical living related donors in recipient groups 3 and 4 (■, ◆) were significantly better ($p < 0.05$) than in groups 1 and 2 (●, ▲).

tioned and were found to have undergone irreversible rejection. During the subsequent years during the follow-up period, the attrition rate of CAD allografts was significantly ($p < 0.05$) greater than that of LRD grafts. Thus at 1 year 80% and 72% of LRD and CAD grafts were functioning, respectively; at 3 years 72% and 60%; at 5 years 68% and 52%; and at 8 years, 68% of LRD grafts continued to function but only 47% of CAD grafts (t½ after 3 months = 26.2 years *versus* 9.2 years, respectively). For the haploidentical LRD grafts, death accounted for virtually all failures after 2 months.

When recipients of primary cadaver allografts were divided into treatment groups, no differences in overall graft survival were noted (Fig. 3A). When LRD recipients were grouped according to treatment protocols, however, a progressive improvement in results was noted in time (Fig. 3b), with grafts in groups III and IV surviving significantly ($p < 0.05$) better than those in groups I and II. However no differences were noted in comparing the results of group III patients who received CyA at an initial dose of 12 mg/kg/day and group IV patients who received triple therapy.

TABLE 3. *Mortality and Morbidity Rates of Allograft Recipients*

Donor	1 Year	1–8 Years	Total
Death			
CAD	20 (5%)	12 (3%)	32 (18%)
LRD	3 (4%)	6 (7%)	9 (11%)
Total	23 (5%)	18 (4%)	41 (9%)
Cause			
Pneumonia	10	11	21
Other sepsis	8	6	14
Misc.*	5	1	6
Complications			
CAD	46 (12%)	16 (4%)	62 (16%)
LRD	4 (5%)	1 (1%)	5 (6%)
Cause			
Pneumonia	26	5	31
Hepatitis	12	1	13
Misc.*	14	10	23

* Includes myocardial infarction and gastrointestinal complications. CAD, cadaver donor; LRD, living related donor.

The actuarial survival rates of primary and secondary CAD grafts were then compared (Fig. 2). The incidence of second or subsequent allografts failing early was significantly ($p < 0.05$) higher than that of primary grafts: at 3 months 80% of primary grafts but only 67% of secondary grafts functioned; at 1 year 75% and 63% functioned, and at 3 years 66% and 54% continued to function, respectively. During the early follow-up period and the years thereafter, however, the relatively parallel rate of attrition between these two groups (t½ after the first 3 months = 9.3 years and 7.4 years, respectively) reinforced the observation that the inferior results of secondary grafts are almost invariably related to rapid immunologic events occurring soon after transplantation. If the grafts functioned beyond the first few weeks, their overall survival for prolonged periods was comparable to primary grafts.

The question arose whether the survival patterns of primary grafts that ultimately failed influenced those of later secondary grafts. The courses of 82 subsequent (second, third, and fourth) grafts were studied. Eleven recipients of multiple grafts received their first grafts in other centers or before the initiation of this series. There were 44 individuals who lost their primary grafts relatively quickly (at less than 6 months); of these, 25 (57%) of the subsequent grafts also were rejected quickly. In contrast, of 38 individuals whose primary grafts had functioned satisfactorily (more than 6 months), 6 (16%) of the secondary grafts were lost quickly, the remainder functioning for prolonged periods. When patients with secondary allografts were divided into treatment groups, no differences were found in the mediocre results of individuals in groups I and II receiving CyA only (Fig. 4). However the results of grafts in patients receiving triple therapy (group V) were significantly ($p < 0.05$) better than the other groups and contrasts to the lack of effect of the triple regimen among recipients of first cadaver grafts (Fig. 3A).

Despite judicious attention to CyA doses and careful maintenance of putatively therapeutic blood levels, 64% of all CAD and 57% of LRD recipients experienced at least one episode of acute rejection within the first 3 months. Indeed the mean number of rejections was 1.5 for all recipients, regardless of donor source (range, 1.4 to 2.0 episodes for recipients of LRD kidneys, 1.2 to 2.4 episodes for patients receiving CAD organs). There were no differences in incidence of rejection when first or subsequent grafts were compared or when treatment protocols including triple therapy were contrasted.

There were seven technical failures in the series, four secondary to renal vein thromboses; two of these occurred in pediatric kidneys. There were two arterial thromboses and one arterial disruption secondary to erosion by an indwelling drain. Ureteral complications, either leak or stenosis, developed in 29 instances (6%), the majority in the first 3 months after engraftment; 24 of these occurred in CAD grafts, five in LRD kidneys. No correlation of this complication and rejection was found. There were 4 instances of renal artery stenosis (1%) that did not become manifest for at least 6 months. All these mechanical complications were repaired successfully.

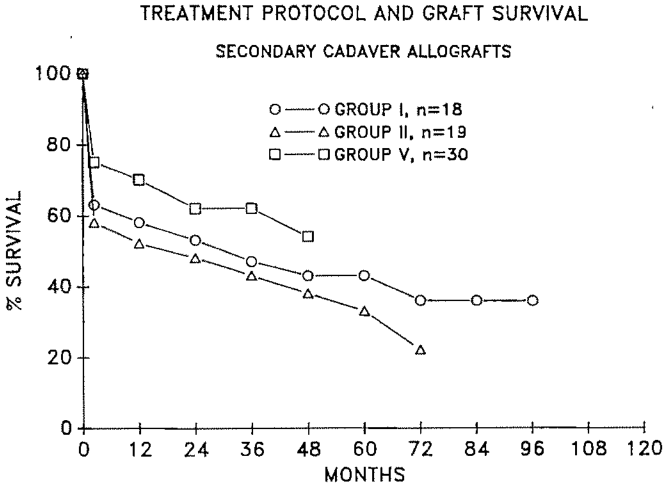


FIG. 4. The triple therapy regimen in recipients of secondary cadaver grafts (group 5) (□) do significantly ($p < 0.05$) better than those in other treatment groups (○, △).

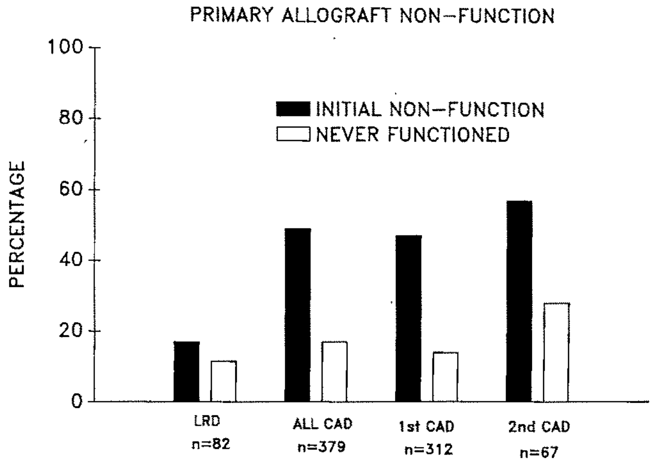


FIG. 5. Primary nonfunction of kidney transplants is noted. The incidence of initial nonfunction among LRD grafts (17%) is significantly ($p < 0.001$) less than among CAD grafts (49%). The percentage of LRD grafts that never functioned (71%) is significantly ($p < 0.001$) higher than that of CAD grafts (34%). Statistical significance was determined by comparing ratios of initial nonfunction to grafts that never functioned (■: □).

Allograft Function

The period of cold ischemia did not vary in time throughout the entire experience. Primary nonfunction occurred in 49% of CAD kidneys and 17% of LRD grafts (Fig. 5). The rate of primary nonfunction was also higher among secondary CAD grafts than primary grafts. Despite initial nonfunction, however, 66% of all CAD grafts recovered without appreciable functional compromise. The rate of immediate function of kidneys from LRD sources was considerably higher (83%). However 71% of LRD grafts experiencing primary nonfunction never functioned at all as compared to 34% of cadaver kidneys; the majority of such organs, regardless of donor source, were found to have experienced rapid and fulminating rejection and almost invariably showed major degrees of both cellular and vascular rejection on biopsy. No differences were apparent between treatment protocol and rate of primary nonfunction.

To determine whether the ultimate function of kidneys that had recovered from initial ATN had become compromised by the insult, levels of serum creatinine were assessed serially between 7 days and 6 years after transplantation in 276 patients whose grafts had diuresed immediately, and compared to 113 who had sustained reversible primary nonfunction. No differences in creatinine levels were noted throughout the entire follow-up period (data not shown). In addition there were no differences in overall graft survival between these two groups.

We then tried to determine whether renal function deteriorated progressively in time among all recipient groups by calculating mean and median serial serum creatinine levels in all available patients. Although individual graft loss after 1 year was almost invariably due to chronic rejection, mean serum creatinine levels of the patients remaining at each time period were remarkably stable and did not change in time (Fig. 6). Similarly the median creatinine levels, although slightly but insignificantly lower than the means at each time interval, did not vary throughout the follow-up period (data not shown).

The relationship of early levels of serum creatinine to eventual graft function also was determined. Patients were divided into those whose serum creatinine at 1 month was more or less than the mean of 2.5 mg/dL and their graft functioning was examined serially up to 8 years thereafter. Interestingly no differences became apparent either in overall renal function or in graft survival of these two groups throughout the entire follow-up period. From these functional data, it appears that the majority of graft loss 1 year or later after transplantation is secondary to specific events (progressive chronic rejection) in a few individual patients rather than from factors such as CyA, which uniformly affect the entire cohort.

Discussion

A decade of experience using CyA as primary immunosuppression in recipients of renal allografts has been reviewed. The mortality and complication rates of graft recipients have remained relatively stable over time in our unit[13,16] and usually occur secondary to infectious processes. However the incidence of both fatal and nonfatal pneumonias in this series may have been skewed by a cluster of cases of Pneumocystis carinii pneumonia developing within a 4-month period; no further cases have developed since routine use of prophylactic trimethoprine-sulfa has been initiated in all recipients once renal function improved toward normal. However, despite better understanding and judicious use of immunosuppression with progressive lowering of drug dosage, the potential for serious septic events still threaten this recipient population. Prompt diagnosis and aggressive treatment of such complications are crucial.

Overall allograft survival within the first year of engraftment, particularly that of kidneys from CAD donors, is less satisfactory in this series than that reported by some individual groups and in some multicenter trials.[17-20] However, when pooled data from large numbers of centers are analyzed, the overall results of CAD grafts do not seem as favorable as generally believed, with about 70% of 0 to 1 HLA-mismatched kidneys and about 60% of those with 5 to 6 mismatches functioning at 1 year[21]; such data may give a more realistic general evaluation of renal transplantation in the 1980s than the results from individual centers in which local reduction of particular risk factors, such as short cold ischemic time, may lead to better graft function.

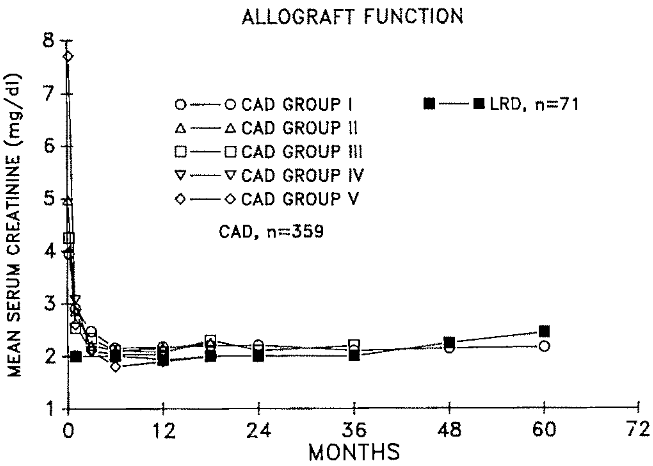


FIG. 6. Patterns of long-term renal graft function in the various treatment groups are analyzed. The majority of late graft loss is secondary to particular events (chronic rejection) in individual patients, not factors (CyA nephrotoxicity) affecting the entire cohort. There are no differences in overall graft function.

It is clear that the comprehensive use of CyA has been effective in improving clinical results over those of azathiaprine,[17-20] particularly as its actions, toxicities, and interactions have become increasingly appreciated.[5] Other factors contributing to this improvement include tissue matching between donor and recipient at the HLA-B and DR loci[22,23] and the availability of newer anti-rejection modalities such as OKT3.[24] Although all patients in this series had received between one and five blood transfusions before transplantation, the beneficial effects of transfusion in CyA-treated patients have become less obvious in recent years, a trend actually beginning before the advent of this agent.[25] In addition the availability of erythropoietin for those with end-stage kidney disease has increasingly precluded the need for transfusion.

Most grafts lost early after transplantation either never functioned or failed within the first few months, almost inevitably from uncontrollable rejection. As shown in Figure 5, primary nonfunction occurred in 49% of all CAD kidneys and in 17% of LRD organs. However this condition was transient in 66% of the CAD kidneys; in contrast 71% of LRD kidneys with primary nonfunction never recovered secondary to early and irreversible rejection. In this series initial nonfunction that reversed itself had no appreciable long-term effects on ultimate renal function and probably represented ATN with or without mild rejection activity. This finding, based on assessment of multiple serial values of serum creatinine, contrasts to the findings of other series that show that long-term function is inferior in kidneys with initial nonfunction.[2]

Most failed grafts are lost within a few days or weeks of transplantation secondary to irreversible rejection, regardless of donor source. The initial dose of CyA made no appreciable difference in the incidence of rejection in this series, as also noted by others.[26] However, unlike previous analyses of our experience with CyA,[13] the results of haploidentical LRD grafts are significantly better than those of CAD kidneys, particularly in the long term (Fig. 1), and are comparable to results from other individual centers.[17-20] Indeed the results with LRD organs show improved survival in time, in contrast to the various treatment groups of CAD recipients. It appears that chronic rejection plays little role in the LRD group because the attrition rate can be accounted for by recipient mortality (Fig. 1)

The mediocre success of secondary CAD transplants has been noted for many years; in the azathioprine era, the 1-year survival rate of such grafts was about 30%, with long-term survival rates decreasing proportionately.[27,28] These results have improved since the use of CyA, although their function still remains 10% to 20% less than that of primary transplants.[13,29,30] Although triple therapy made no appreciable difference in improving survival rates of primary grafts, its use in recipients of secondary grafts may be more effective, as suggested in this study. Human lymphocyte antigen (HLA) matching, particularly at the DR locus, also may be important.[21] Risk factors contributing to loss of the retransplanted organs have been thought to include recipients who are older (particularly male patients), diabetics, early rejection of the first graft, retransplantation within 6 months, and high levels of panel reactive antibodies.[17,29] The increased potential for second graft loss in recipients who had rejected their first grafts in less than 6 months was confirmed in this study. Clearly the inferior results in this group of recipients remain an area of clinical transplantation that deserves emphasis in the future.

As in the period between the mid-1960s and early 1980s, when azathioprine was used as primary immunosuppression, the overall results of graft survival in recipients treated with CyA have stabilized despite increasing understanding of rejection and the gradual availability of monoclonal antibodies to modulate various steps in the process. The concept of combination therapy to improve results and decrease CyA nephrotoxicity also has evolved, particularly as it has become clear that several pharmacologic agents, including other immunosuppressive modalities, may interact with CyA in an additive or even synergistic manner.[15] Routine conversion to azathioprine 3 to 6 months after transplantation has been reported; however, despite discontinuation of CyA, renal function improved but never normalized.[10,11] We have continued to use CyA for long-term maintenance in progressively decreasing doses to levels of 2 to 3 mg/kg/day. Triple drug therapy (CyA, azathioprine, and prednisone) or quadruple therapy (with the addition of antilymphocyte globulin) has been used with varying degrees of enthusiasm, while sequential immunosuppression has been tried to obviate the early nephrotoxic effects of CyA on kidneys potentially damaged by prolonged ischemia and storage.[7-9] Overall the results using triple therapy are better than those of azathioprine and steroids alone and are comparable to those of CyA and steroids.[31] Toxicity may be higher, however,[32] and CyA nephrotoxicity may not be importantly reduced despite a lower dose of the drug.

The long-term results of kidney transplantation in the CyA era also may not be as satisfactory as originally hoped. Ideally engraftment should be more than a brief interlude in a chronic disease; indeed sustained long-term function should be expected. However CAD grafts functioning well at 1 or 2 years will rarely continue indefinitely, with eventual graft loss usually occurring secondary to chronic rejection. The attrition rate of LRD grafts is dependent on recipient mortality. In a longitudinal analysis of 14,005 kidney recipients transplanted before 1976 in the azathioprine era and followed thereafter, graft survival at 10 years was 67% in HLA-identical LRD grafts, 38% among those from parents and 20% of transplants from CAD sources.[21] Similarly, in a current analysis of projected 20-year graft survival rates for the CyA era, it appears that the attrition rate after 1 year is no different than in those individuals treated initially with azathioprine[33]; much of the late graft loss is on an immunologic basis because a clear-cut HLA-matching effect is seen. Our follow-up data, although not yet extending beyond a decade, seem to corroborate these suggestions; chronic rejection remains a conundrum that has not been solved.

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Delayed Presentation and Treatment of Popliteal Artery Embolism

RICHARD P. CAMBRIA, M.D., BRIAN A. RIDGE, M.D., DAVID C. BREWSTER, M.D., ASHBY C. MONCURE, M.D.,
R. CLEMENT DARLING, M.D., and WILLIAM M. ABBOTT, M.D.

From the Surgical Service of the Massachusetts General Hospital, and the Department of Surgery, Harvard Medical School, Boston, Massachusetts

In the course of reviewing a 10-year experience with popliteal artery embolism (PAE), two distinct patterns of clinical presentation were identified. In addition to those patients presenting with typical acute (symptom duration less than 7 days) arterial ischemia, a second group was identified who presented with more chronic symptoms. The present study was conducted to contrast the clinical factors and treatment of these two temporal patterns of presentation with PAE. Sixty PAEs in 58 patients were documented by the combination of angiography and/or exploration of the popliteal artery. Acute presentation (AP) was seen in 41 (68%) of these and delayed presentation (DP) was noted in 19 (32%) patients. Delayed presentation patients typically presented with a history of sudden onset of claudication or rest pain and a median symptom duration of 30 days. Eighty per cent of AP patients presented with immediately threatened limbs. Angiography was generally diagnostic of chronic popliteal embolism. In the acute group, 90% were treated with embolectomy alone, while 20% of the DP group required bypass grafting. However in two thirds of the DP group, embolectomy alone performed through a direct popliteal approach was possible. Current results with overall limb salvage (92%) and mortality (7%) represents a substantial improvement compared to the authors' previous experience with PAE. The current study suggests that as many as one third of patients with popliteal artery embolism may present in delayed fashion with chronic symptoms. Furthermore most of these patients can be treated with direct popliteal embolectomy alone with favorable results.

EMBOLISM TO THE popliteal artery traditionally has been associated with an acute presentation and significant risk of limb loss.[1-7] Corroborative data are available from the early experience with popliteal artery trauma, wherein acute injuries, treated by ligation, resulted in limb loss in 67% of cases.[8] In a previous report, we noted a 76% limb salvage rate in patients with surgically treated popliteal artery embolism, which was distinctly inferior to the results seen with more proximal iliofemoral emboli.[1,6] Furthermore inadequate or incomplete surgical embolectomy frequently was responsible for the inferior results seen with popliteal embolectomy when compared to femoral embolism. That experience prompted a recommendation for the liberal use of a direct popliteal artery approach and has been accompanied by the recognition of two distinct patterns of clinical presentation in patients with popliteal embolism. To define the incidence and management of these varied clinical presentations, we reviewed a 10-year experience with PAE, encompassing the period since our last review of this topic.

Methods

All hospital and office records of the authors' patients with a proved diagnosis of popliteal artery embolism treated between January 1978 and December 1988 were reviewed with an emphasis on the temporal pattern of presentation. The diagnosis was confirmed by angiography and/or operation in all but three patients (two refused revascularization and one was considered too unstable for angiography or embolectomy). Patients found to have acute or chronic atherosclerotic thrombosis, and those in whom the diagnosis of embolism was equivocal, were excluded. Patients were divided into two groups based on the duration of ischemic symptoms at time of hospital presentation. Acute presentation (AP) was defined as symptoms of arterial ischemia of less than 7 days duration and delayed presentation (DP) as symptom duration more than 7 days. Patient demographic and clinical character-

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Address reprint requests to Richard P. Cambria, M.D., Massachusetts General Hospital, 15 Parkman St., Suite 458, Boston, MA 02114.

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istics and diagnostic and treatment modalities were recorded.

Results

Sixty popliteal artery emboli (PAE) were identified in 58 patients. Acute presentation was seen in 41 patients (68%), with a median symptom duration of 12 hours (range, 2 hours to 6 days). Delayed presentation was noted in 19 patients (32%), with a median symptom duration of 30 days (range, 7 to 120 days). The average age was 66 years in the DP group and 62 years in the AP group. No significant differences in cardiovascular risk factors between these two groups (hypertension, smoking history, diabetes, heart disease) were found. Table 1 lists the etiology of emboli in the two groups.

Minor gangrene was present in three of the late (DP) group, and the remaining patients had a history of sudden onset of claudication or rest pain, which was presumed to represent the embolic event. Eighty per cent of the AP group were thought to have immediately threatened limbs requiring emergent intervention. Table 2 lists the presenting symptoms and physical findings.

In the DP group, the average ankle to brachial index (ABI) was 0.32 (claudicators, 0.44; rest pain, 0.27; and gangrene, 0.13). In the AP group, few patients had ABI determination; most patients presented with acute ischemia, with a clinical diagnosis of popliteal embolism, and did not have noninvasive laboratory studies. Patients who presented with weeks or months of symptoms underwent angiography. Thus angiography was performed in 18 of 19 patients (one refused) with DP and provided the correct diagnosis in 15 of 18 patients. Arteriograms of the other three patients were interpreted as atherosclerotic popliteal occlusions subsequently disproved at surgery. Two of the three had emboli from atherosclerotic disease in the proximal femoral artery. This proximal disease in association with popliteal occlusion led to the incorrect diagnosis of atherosclerotic occlusion.

TABLE 1. *Etiology of Popliteal Artery Embolism*

Etiology	Delayed Presentation n = 19 (%)	Acute Presentation n = 41 (%)
Cardiac		
Atrial fibrillation	6	9
Recent MI	3	2
LV aneurysm		1
Endocarditis		1
Coronary surgery		1
Coronary artery disease	4	8
Total	13 (68)	22 (54)
Noncardiac		
Proximal atherosclerosis	2	9
Aortic aneurysm	2	2
Aortic graft	2	1
Total	6 (32)	12 (29)
Iatrogenic		
Aortic balloon pump		4
Aortic valvuloplasty		1
Angiography		1
Intra op aortic surg		1
Total		7 (17)

MI, myocardial infarction; LV, left ventricular.

TABLE 2. *Presenting Symptoms*

Symptom	Delayed Presentation n = 19 (%)	Acute Presentation n = 41 (%)
Claudication	9 (47)	0
Rest pain	7 (37)	8 (20)
Acute severe ischemia*	0	33 (80)
Toe gangrene	3 (16)	0

* Loss of sensory or motor function, or evidence of muscle compromise.

The technical aspects of surgical treatment are detailed in Table 3. Ninety per cent of the AP group were treated with embolectomy alone, while 4 of 19 patients in the DP group required femoral–popliteal or femoral–tibial bypass grafting. Bypass grafts were used either because proximal superficial femoral artery lesions were treated concurrent with the popliteal embolism or because of inability to remove the organized thrombus in the popliteal artery. However two thirds of the DP group were treated with embolectomy alone performed *via* a direct popliteal approach. While popliteal artery exploration was indicated to remedy the situation, the operative findings were critical to establishing the correct pathologic diagnosis in all but 3 of 19 DP patients. These three patients presented with claudication and arteriograms revealed normal proximal and distal vasculature in association with a known cardiac source for embolism. In the remaining DP patients, operative exploration revealed a densely adherent thrombus in the popliteal artery lumen, but the vessel wall itself was either free of or afflicted with only minor atherosclerosis.

Treatment results in the present series are shown in Table 4. One amputation was required in a DP patient

TABLE 3. *Treatment*

Treatment	Delayed Presentation (n = 19)	Acute Presentation (n = 41)
Embolectomy		
Femoral approach	1	8
Popliteal approach	11	30
Embolectomy and bypass	4	0
Anticoagulation only	3	2
Amputation only	0	1

TABLE 4. *Results*

Result	Delayed Presentation (n = 19)	Acute Presentation (n = 41)
Limb salvage	95%	90%
Death	0%	10%

who had a 6-week history of rest pain that became worse 1 day before admission (diagnosis of popliteal embolus was confirmed at operation and a femoral embolus occurred on the opposite side during the same hospital stay). The three patients in the DP group who presented with minor gangrene healed after successful revascularization. Four amputations were required in AP patients, one in a patient who refused embolectomy and three in patients treated with popliteal embolectomy after prolonged periods of preoperative ischemia. There were no deaths in the DP group and four deaths in the AP group, all secondary to coexisting coronary artery disease.

Data from our previous reports[1,6] is compared to the overall results of the present series in Table 5. While statistical comparisons are not valid due to some overlap of the review periods, the increased use of a direct approach to the popliteal artery appears to be associated with a trend toward improved limb salvage and declining mortality rates.

Comment

It is generally accepted that early intervention is warranted in patients with popliteal embolism and a threatened limb. Elliot[9] documented the increase in ischemic complications in patients with arterial emboli in whom preoperative delay exceeded 8 hours. He also noted a decrease in complication rate in those in whom preoperative delay was longer than 7 days. He attributed this to a 'harvesting effect.' (Those with severe ischemia will have died or lost their limb by this time.) Despite several reports in the literature, it is less well appreciated that some patients with arterial embolism who present late can be treated successfully with embolectomy.[9–16] Olwin in 1953 reported successful embolectomy in four of six patients who presented 22 hours to 3.5 months after embolism.[10] In 1959 Haimovici reported 10 cases of late embolectomy (13 hours to 20 days), including four popliteal emboli, two of which were treated successfully with delayed embolectomy.[11] However the earlier literature[10–14] generally considered 'delayed' embolectomy to be that carried out 8 to 12 hours or more after the presumed onset of symptoms. In fact these reports antedated introduction of the Fogarty embolectomy catheter. Two more recent reports emphasized that successful delayed embolectomy is sometimes feasible even weeks to months after the onset of embolism,[15–16] yet even when considered together they contain only a few patients with popliteal embolism.

The etiology of PAE was similar in the two groups of patients. Cardiac sources continue to be the most common etiology of PAE. In an earlier series from our institution,[17] atrial fibrillation alone accounted for 70% of all peripheral emboli while in this series only 25% of patients had atrial fibrillation alone without evidence of ischemic heart disease. This reflects, in part, the decreased incidence of rheumatic heart disease but also the nature of the authors' practice, which includes many patients with both proximal atherosclerotic disease, and previously placed grafts as sources of emboli. Iatrogenic emboli occurred in patients under observation and therefore are all identified early. If this group is eliminated, the incidence of delayed presentation would be even higher.

When examined from the perspective of documented popliteal embolism, we found that as many as one of three patients with PAE will present in delayed fashion, several days to weeks after the event. It is our suspicion that such cases frequently are not recognized as being embolic in origin, but rather are treated under the assumption that they represent the sequelae of atherosclerotic occlusive disease. The diagnosis is suggested by the abrupt onset of claudication or rest pain. Differences in collateral circulation, and/or the extent of propagating thrombus, must explain the variation in ischemia experienced by those who present acutely and those with delayed presentation. However no difference in the incidence of clinically evident atherosclerotic vascular disease was found between the two groups, and we intentionally excluded patients with documented or suspected (after treatment) atherosclerotic thrombosis.

As we have noted previously, the distinction between embolic and thrombotic occlusion can be problematic,[18] particularly in an era when the demographic and clinical characteristics of patients so affected are quite similar.[6] Yet, as we and others have emphasized, such distinction is important because the management and anticipated treatment results vary significantly between embolic and thrombotic occlusion producing acute lower extremity ischemia.[18, 19] The diagnosis often depends on complete

TABLE 5. *Surgical Treatment of Popliteal Embolism*

Treatment/Result	MGH, 1964–1980[6] (n = 67)	Present Series (n = 60)
Initial embolectomy *via* direct popliteal approach (%)	31%	83%
Limb salvage	76%	92%
Death	22%	7%

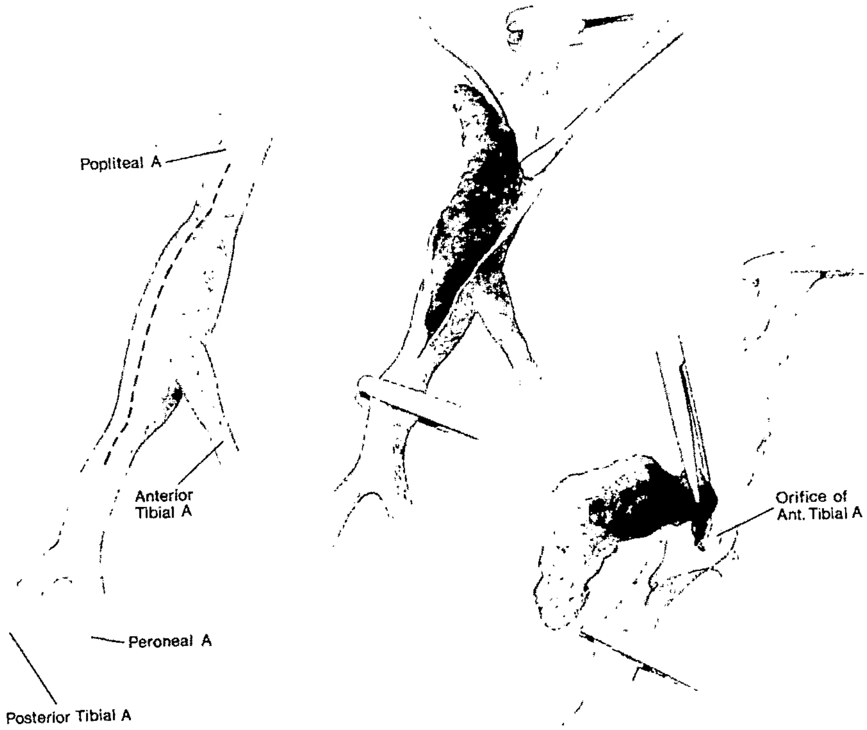

FIG. 1. Direct exposure of popliteal artery allowing longitudinal arteriotomy, embolectomy assisted by endarterectomy spatula, and direct visualization of orifice of anterior tibial artery.

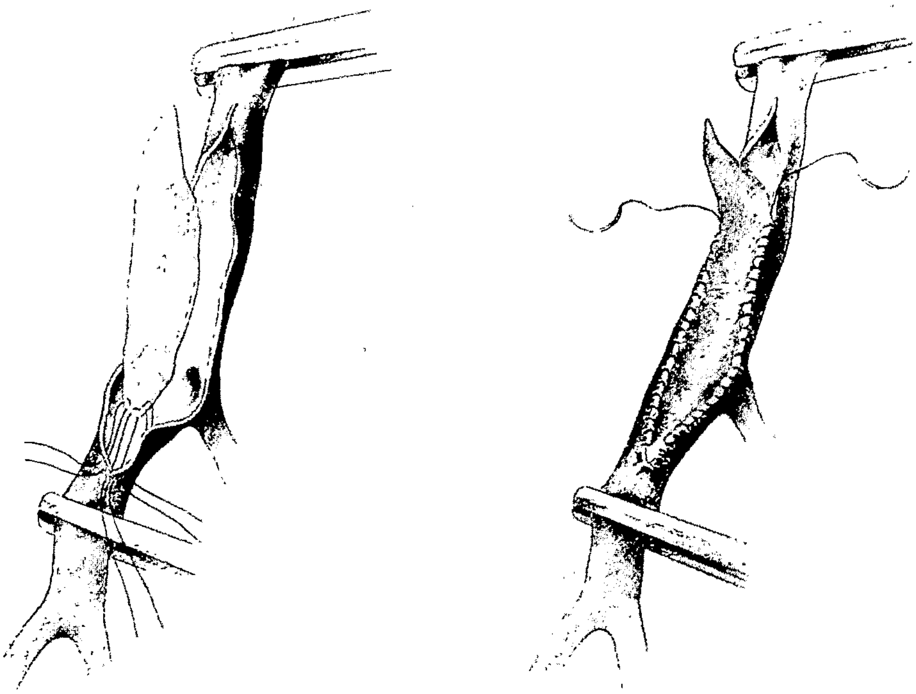

FIG. 2. Closure of arteriotomy with saphenous vein patch using interrupted mattress technique at distal end of arterotomy.

angiography, and in accordance with their chronic symptom development, patients presenting in delayed fashion underwent arteriography. Patients in whom a clinical diagnosis of acute PAE is made routinely are treated with prompt embolectomy. In this series most diagnoses were proved by operative exploration, the ultimate diagnostic test, but angiography generally was diagnostic in the DP group. The angiographic findings supportive of the diagnosis of embolism are (1) convex filling defect in the vessel lumen with abrupt occlusion, (2) poorly developed collateral vessels, (3) lack of associated atherosclerotic disease in adjacent segments of artery, and (4) often found at bifurcations.[20,21] A high index of suspicion in patients with the history of acute onset of symptoms (regardless of duration) coexisting with the above angiographic findings should increase diagnostic accuracy and prompt the surgeon to consider direct popliteal artery exploration rather than proceeding initially with distal bypass grafting.

Our approach to patients with PAE has been described previously.[22] We prefer direct exploration of the popliteal artery if a pulse is present in the popliteal fossa. This is particularly helpful in cases in which the embolism has been present for a prolonged period. Brock, in his 1962 review of late arterial embolectomy, thought the procedure might be more correctly described as 'thromboendarterectomy.'[12] Indeed the technical aspects of such delayed embolectomy are worthy of comment. Catheter embolectomy from a remote site (femoral artery) seldom will be feasible because the organizing embolism has engendered an adherent inflammatory response in the vessel wall. Removal of such chronic emboli requires direct exposure and a sharp dissection off the vessel wall in the manner of an endarterectomy (Fig. 1). It has nearly always been possible to establish the appropriate plane of dissection between the thrombus and the vessel wall; dissection in a true endarterectomy plane (within the vessel media) is to be carefully avoided because it may jeopardize the patency of the outflow tract vessels. Complete visualization of the embolism and its adherent thrombus mandates a longitudinal arteriotomy, generally carried beyond the origin of the anterior tibial artery and usually closed with a vein patch (Fig. 2). Two thirds of our patients with DP of PAE were treated with a direct popliteal embolectomy, with only 4 of 19 patients requiring a bypass procedure. Those requiring bypass procedures had either some associated atherosclerotic disease of the superficial femoral artery, or the propagating proximal thrombosis was sufficiently organized to preclude easy removal. In these cases short segment vein graft bypasses were used. Adequacy of all reconstructions are assessed by intraoperative pulse volume recording and/or completion angiography. In selected cases intraoperative angioscopy has proved useful in verifying the technical results of reconstruction. It seems likely that this modality will, in time, diminish the use of operative angiography.[23]

In earlier reports dealing with popliteal emboli, we noted both limb loss and death in 25% of patients.[1,6] We speculated that many treatment failures resulted from incomplete embolectomy of the popliteal and tibial vessels when the procedure was done from a 'remote' arteriotomy in the femoral artery. Our more recent experience with popliteal emboli detailed herein reflects frequent use of a direct popliteal artery approach and has been accompanied by improved results with respect to both limb salvage and overall mortality. Limb salvage was more than 90% in currently treated patients with both acute and delayed presentation of popliteal emboli. While the improvement in limb salvage is attributed to the routine use of a direct popliteal approach for all patients with embolic occlusion at that level, other factors are probably involved in the improved overall survival. Patients with chronic embolism often fit noninvasive lab criteria for rest pain and although the presenting symptom may be claudication alone, we advocate prompt exploration because the reconstruction required is generally quite simple. Furthermore delay in revascularization can result in progressive loss of outflow tract vessels, hemodynamic deterioration, and the subsequent requirement for more distal bypass. If the diagnosis of chronic PAE is suspected, angiography is indicated to verify the diagnosis. This has obvious implications for patient treatment, regardless of whether revascularization is indicated because permanent anticoagulation is indicated in patients with PAE.[9] These favorable results suggest that a direct surgical approach should be considered in all patients with acute or chronic popliteal artery embolism.

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Plasma Gastrin and Cholecystokinin Response After Pylorus-preserving Pancreatoduodenectomy with Billroth-I Type of Reconstruction

AKIRA TANGOKU, M.D., MASAHIRO NISHIKAWA, M.D., ATSUSHI ADACHI, M.D., and TAKASHI SUZUKI, M.D.

Plasma gastrin and cholecystokinin (CCK) responses were measured after a pancreatoduodenectomy (PD) using the Billroth-I type reconstruction combined with distal partial gastrectomy (standard PD) and combined with preservation of the pylorus and the duodenal bulb (PPPD). Six unoperated patients, 4 men and 2 women, were studied as control subjects. Basal plasma levels of gastrin were significantly higher in controls than in patients who had a standard PD ($p < 0.05$) and gastrin responses to a meal were also blunted in these patients. In contrast basal and postprandial levels of gastrin after PPPD were significantly higher than these found in patients with standard PD ($p < 0.05$). Postprandial gastrin response after PPPD were similar in pattern to these found in controls. Integrated gastrin release after PPPD was less than that of the control but was significantly greater than that in patients with standard PD. Basal plasma levels of CCK in the patients after the standard PD were significantly lower than in controls and significantly higher postprandial levels of CCK were found after PPPD compared to standard PD ($p < 0.05$). However integrated CCK from 0 to 120 minutes were not significantly different between PPPD and standard PD groups. Based on these observations concerning hormonal release of gastrin and CCK, preservation of the stomach and the duodenal bulb appears to be a more physiologic reconstructive procedure than the standard PD. In addition the operation probably has more beneficial effect on the injured pancreas in time.

From the Department of Surgery II, Yamaguchi University School of Medicine, Ube, Japan

GASTRIN AND CCK are the important gut hormones that have pancreatotrophic action and an important role in the regulation of exocrine and endocrine function of the pancreas.[1-5] Pylorus-preserving pancreatoduodenectomy (PPPD) is a surgical procedure of reconstruction that preserves the gastrointestinal function after a pancreatoduodenectomy (PD).[6] We performed a new type of reconstruction[7] in which we resected a minimal area of duodenum preserving the neurovascular supply of the pylorus and the duodenal bulb (PPPD).

Gastric acid release, gastric emptying, and marginal ulcer formation have been reported after PPPD.[8-11] However there are few reports concerning hormonal changes in gastrin and CCK in these PPPD patients. These hormones accelerate functional and morphologic regeneration of the residual pancreas when comparisons are made before and after PD. In this study we compared basal and postprandial levels of gastrin and CCK between PPPD, standard PD group, and control.

Materials and Methods

From April 1987 through August 1989, 33 patients underwent PD procedure in our department, of whom PPPD was done for 15 patients and the standard PD was for 18 patients. Both PPPD and standard PD were reconstructed with the Billroth-I method (Fig. 1). Seven patients (3 men, 4 women), ranging in age from 34 to 72 years (mean, 48.8 years) who had the PPPD were examined. The final diagnosis of these patients was chronic pancreatitis in 2, common bile duct cancer in 2, pancreatic cyst in 2, and islet tumor in 1. Six of the patients (1 men, 5 women) ranging in age from 42 to 75 years (mean, 56.7 years) who had undergone standard PD also were examined. This final diagnosis for them was five with pancreatic head cancer and one with common bile duct cancer. These patients were free of recurrence of disease at the time of the study.

The control group consisted of six patients (four men, two women), ranging in age from 18 to 75 years (mean, 40.3 years). Three of them suffered from chronic pancreatitis, one with congenital pancreatic cyst, and two were normal volunteers. They had not been operated on before this study. A test meal was given to PPPD patients 1 to 11 months (mean, 5.1 months) and to PD patients 2 to 5 months (mean, 4.1 months) after surgery.

After an overnight fast, a catheter was inserted into a peripheral vein to collect blood samples. Each patient was

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Address reprint requests to Akira Tangoku, Department of Surgery II, Yamaguchi University School of Medicine, 1144 Kogushi, Ube 755, Japan.

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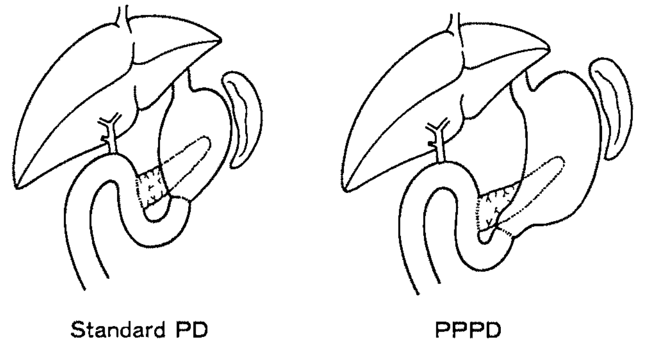

FIG. 1. Schema of two types of reconstruction with Billroth-I method after pancreatoduodenectomy.

given 250 mL of a liquid meal (YH-80 consisting of 7.75 g of protein, 6.5 g of fats, and 40.35 g of glucose, Meiji Milk Products Co., Ltd., Tokyo, Japan), and blood samples were collected for gastrin and CCK assays at 10-minute intervals before and for 120 minutes after the meal.

Blood Collection

Blood samples were collected with or without heparin (10 U/mL) and Trasylol (50 KIU/mL), maintained on ice, and plasma separated by centrifugation at 4°C and 3000 rpm for 15 minutes. Plasma was stored at −80°C for subsequent assay of gastrin and CCK by radioimmunoassay (RIA).

Radioimmunoassays

Gastrin. Blood samples were collected without heparin and plasma separated by centrifugation. Plasma gastrin was measured using a CIS gastrin radioimmunoassay kit (Gastrin RIA Kit II, Dinabot, Japan).

CCK. Blood samples for CCK were collected in tubes containing 10 U/mL of heparin and 50 KIU/mL of Trasylol. Plasma concentration of CCK was measured by radioimmunoassay using a specific antibody (specific to CCK-33 [100%], CCK-8 [100%], CCK-39 [84.6%], gastrin [0%]). The details of this method has been published previously.[12,13]

Dual Scintigraphy

During a 12-hour fast, the patients who had PD (standard PD or PPPD) were positioned semirecumbent in front of a large field of view gamma camera (Digital gamma camera GCA-A/W2, Toshiba, Tokyo, Japan) fitted with a diverging collimator (Toshiba medium energy general purpose collimator model ROC-930A) on line to a scintigraphic data analyzer (Toshiba medical image processor model GMS-550J). One hundred eighty-five MBq of ^{99m}Tc-N Pyridoxyl-5 methyl tryptophan (^{99m}Tc-PMT) was administered from the peripheral vessel before each patient was given 60 g of semiliquid meal containing 10 g of Clinimeal (Eisai Co., Tokyo, Japan) labeled with 37 MBq of ^{111}In-diethylene triaminepentaacetic acid (^{111}In-DTPA). Both agents were imaged by using the two windows on the gamma camera by every 1 minute for 45 minutes and all data were stored in computer. Time activity curve was calculated by counting the percentage radioactivity of maximum in the area of the upper small intestine.

Statistical Evaluation

The results are expressed as mean ± SE, and ANOVA with Newman-Keuls test and independent student t test (for comparison of the integrated values) were used to evaluate statistical significance. A probability value of less than 0.05 was considered significant.

Results

Measurement of Plasma Levels of Gastrin

Postprandial plasma gastrin responses in control patients after standard PD and after PPPD are shown in Figure 2. In standard PD patients, the basal level of gastrin was significantly lower than the control ($p < 0.05$), but there was no significant difference between PPPD patients and the control. In controls plasma gastrin increased from a mean fasting value of 70.1 ± 11.8 pg/mL to 130.7 ± 23.5 pg/mL at 20 minutes after the meal. In the post-PPPD patients, plasma gastrin also increased from 48.2 ± 5.9 pg/mL to 76.6 ± 10.2 pg/mL at 20 minutes after the meal. There was little or no postprandial gastrin release in the standard PD patients and gastrin levels were significantly less than PPPD patients and control throughout the entire time course ($p < 0.05$). Integrated gastrin release (0 to 120 minutes) in the control was 3267.7 ± 1062.6 pg/min/mL, 2092.1 ± 500 pg/min/mL in the PPPD group and 290.4 ± 122 pg/min/mL in the standard PD group, a value significantly less than that of the control and of

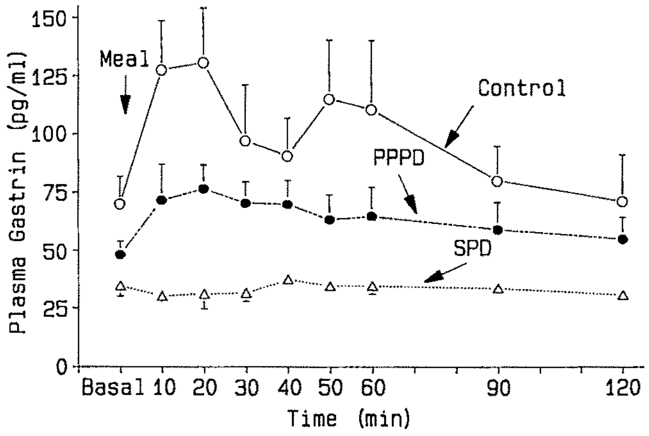

FIG. 2. Comparison of gastrin response after the test meal in control group (n = 5) to standard PD (n = 6) and PPPD (n = 6) procedures. Data are expressed as mean ± SE. There was a significant difference ($p < 0.05$) between standard PD and PPPD groups throughout the time course.

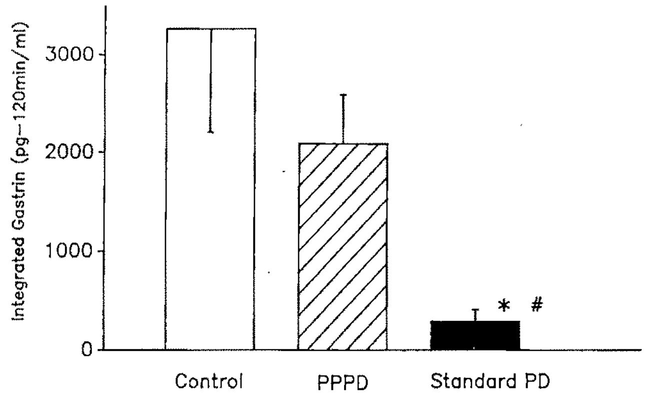

FIG. 3. Integrated gastrin (120 minutes) in control, standard PD, and PPPD groups. Data are expressed as mean ± SE. Asterisk indicates a significant difference ($p < 0.05$) between standard PD and PPPD groups. Sharp sign indicates a significant difference ($p < 0.01$) between standard PD and control.

PPPD patients. There was no significant difference between PPPD and control (Fig. 3).

Measurement of Plasma Levels of CCK

Basal CCK level was 7.0 ± 1.0 pg/mL in the control group, 7.0 ± 2.2 pg/mL in the PPPD group and 1.2 ± 0.8 pg/mL in the standard PD group. Peak response of CCK was 24.0 ± 13.3 pg/mL in the control group at 20 minutes, 15.1 ± 3.4 pg/mL in the PPPD at 30 minutes, and 8.0 ± 4.8 pg/mL in the standard PD at 30 minutes. All peak concentrations were significantly higher than the basal levels ($p < 0.05$).

Basal and postprandial CCK levels in the standard PD group were significantly lower than the control group ($p < 0.05$), whereas postprandial CCK levels were significantly higher in the PPPD group as compared to the standard PD group ($p < 0.05$) (Fig. 4). Cholecystokinin release pattern in PPPD resembled the control and no significant difference between PPPD and control was found.

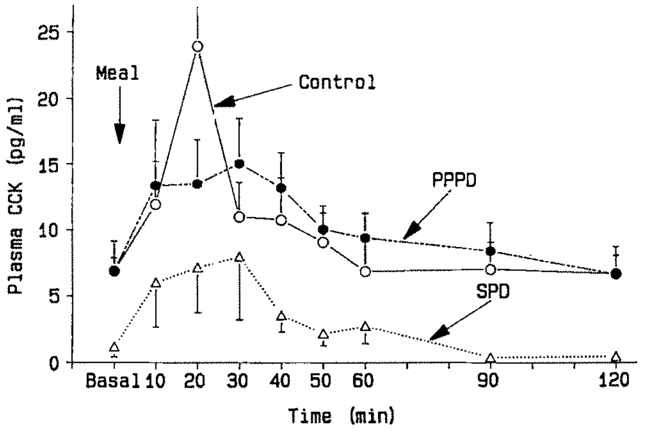

FIG. 4. Comparison of CCK response to the test meal in control (n = 5), standard PD (n = 5), and PPPD (n = 5) groups. Data are expressed as mean ± SE. There was a significant difference ($p < 0.05$) between standard PD and PPPD groups through the entire time course. No significant difference was found between PPPD and control.

The integrated CCK response from 0 to 120 minutes was 390.3 ± 225.6 pg/min/mL in the control group, 424.8 ± 157.5 pg/min/mL in the PPPD, and 267.1 ± 92.6 pg/min/mL in the standard PD group. There was no significant difference between the control, PPPD, and standard PD patients (Fig. 5).

Dual Scintigraphy

Dual scintigraphy with ^{99m}Tc-PMT- and ^{111}In-DTPA-labeled food showed complete and gradual mixing of bile and food in both standard PD and PPPD (Fig. 6). Standard PD showed slightly incomplete mixing of bile and food, but this difference was not found significant.

Discussion

Pancreatoduodenectomy is a procedure that resects the gallbladder, the distal part of the stomach, the entire duodenum, the proximal jejunum, and the head of the pancreas. These portions contain many different kinds of gut hormone-releasing cells, which have important roles in the regulation of gastric acid secretion and metabolism of glucose, protein, and fat. Traverso and Longmire[6] suggested that PPPD with Billroth-II type reconstruction could prevent dumping and other postoperative symptoms as well as maintain weight gain. We[7] performed a new PPPD with Billroth-I type reconstruction and reported that this operative procedure has several advantages over others in that it preserves gut hormonal status.[9]

In the current study we found that fasting plasma gastrin levels were highest in the control and lowest in the standard PD group and that PPPD patients had significantly higher gastrin levels than standard PD. The postprandial gastrin response pattern after PPPD resembled that of the control, as has been reported previously.[9] It is of interest that plasma levels of gastrin in standard PD did not in-

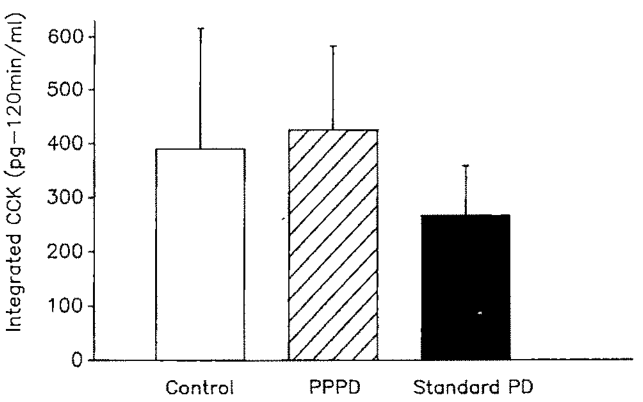

FIG. 5. Integrated CCK (120 minutes) in control, standard PD, and PPPD groups. Data are expressed as mean ± SE. No significant differences were found among control, PPPD, and standard PD groups.

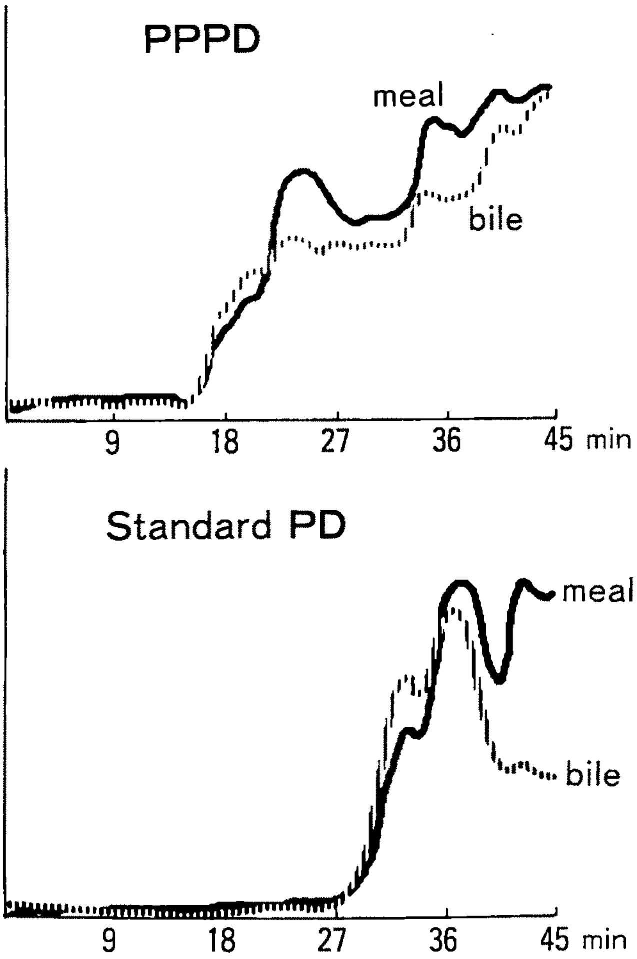

FIG. 6. Time activity curves of dual scintigraphy. Time activity curves were calculated by counting the percentage of maximam radioactivity of ^{99m}Tc-PMT and ^{111}In-DTPA. They showed complete mixture of bile and food both in PPPD and standard PD. No significant difference was found between them.

crease after a meal. Sato et al.[14] also reported that plasma gastrin levels of the patients with Whipple's reconstruction after PD were remarkably reduced after the surgery. Because the normal antrum and bulb are richly populated with gastrin-releasing cells, our results and those of others that show low levels of gastrin after PD suggest that this postoperative phenomenon may be due to the lack of hormone-releasing cells.

Total CCK release in PPPD patients was significantly larger than that of patients with standard PD, whereas postprandial response of CCK was similar to that found in controls. Several factors in PD patients may play a role in alternation of plasma CCK levels. Gastrectomy is one of them. It was reported that patients with gastrectomy, especially in Billroth-II anastomoses, have an impaired release of CCK[15] and an increased incidence of gallstones.[16] Satake and colleagues[17] measured endogenous CCK release after a test meal in controls and in patients with Billroth-I and Billroth-II anastomoses after subtotal gastrectomy and reported that 2 months after the surgery postprandial plasma levels of CCK were similar to the controls. Hopman[18] showed that the mean fasting plasma CCK in patients after gastrectomy was similar to that in controls; however CCK responses to oral fat in the patients after gastrectomy were significantly greater than the control patients. Becker et al.[19] also reported that basal CCK concentrations were not altered after Billroth-I or Billroth-II gastric resections but they reported that a significant increase in plasma CCK levels immediately after food. It has been suggested that the increase in CCK levels after gastrectomy was due to vagotomy and rapid gastric emptying, which results in activation of a large number of food-stimulated CCK cells.[17,18] However Guzman and colleagues[20] and Fried and colleagues[21] found that CCK release is not under vagal control. Thus the effect of gastrectomy to CCK release is still controversial.

Pancreatoduodenectomy is the procedure that resects gallbladder, duodenum, and the upper part of jejunum and stomach. Postprandial CCK levels in the standard PD group in this study were significantly lower than in the controls and PPPD group. Ogden et al.[22] reported that the concentration of CCK-releasing cells is highest in the duodenum and progressively diminishes distally in the small intestine. Lower CCK levels after PD may be due to resection of the duodenum and jejunum, where the concentration of CCK releasing cells is richest.

Basal and postprandial CCK levels were significantly higher in PPPD than in standard PD patients. It is probable that this is due to a good mixture of food with gastric, bile, and pancreatic juice. Gradual duodenal and jejunal acidification releases CCK[23] *via* gastric acid production, which is reported to be normal in PPPD patients (unpublished data). Further evidence that acid might be involved is that secretin levels increase after a meal in the PPPD (data not shown); this is important because, to our knowledge, the only known physiologic stimulus for secretin release is duodenal acidification.

However, even after standard PD, CCK was released well postprandially and no significant difference was found in the integrated CCK between control, PPPD, and standard PD.

In a previous study, we compared CCK release between two types of anastomosis of remnant alimentary tract after anterectomized PD.[24] The plasma CCK response to an oral fatty meal was significantly greater in patients who had a PD with Billroth-I type reconstruction as compared to patients with the Billroth-II type, suggesting that Billroth-I type of reconstruction is a more physiologic reconstruction for the remnant alimentary tract. In this study the patients had Billroth-I type reconstructions both in PPPD and standard PD group.

Patti[25] showed normal gastric emptying of radiolabeled solid food after PPPD using a scintillation camera. We used a double-isotope technique to see the bile and food mixing at the same time and found complete and gradual mixing of bile and food after both standard PD and PPPD.

Our results indicate that postprandial plasma gastrin response in PPPD patients, but not PD patients, resembled that of control. In addition preserving the pylorus and the duodenal bulb as well as Billroth-I type of anastomosis between the bulb and the fourth portion of the duodenum allowed gradual transportation of food and mixture with digestive juice that enhanced CCK release before and after the meal. Gastrin and CCK release in PPPD patients may have a beneficial effect on the injured pancreas in time.

Acknowledgments

The authors thank Otsuka Assay Laboratory, who supported the gastrin and CCK assay; Professor Phillip L. Rayford, of the University of Arkansas for Medical Sciences, Little Rock, AR; Dr. Robert D. Skinner and Michael Webber for their many suggestions in preparing the manuscript; and Mae Agnew for typing the manuscript.

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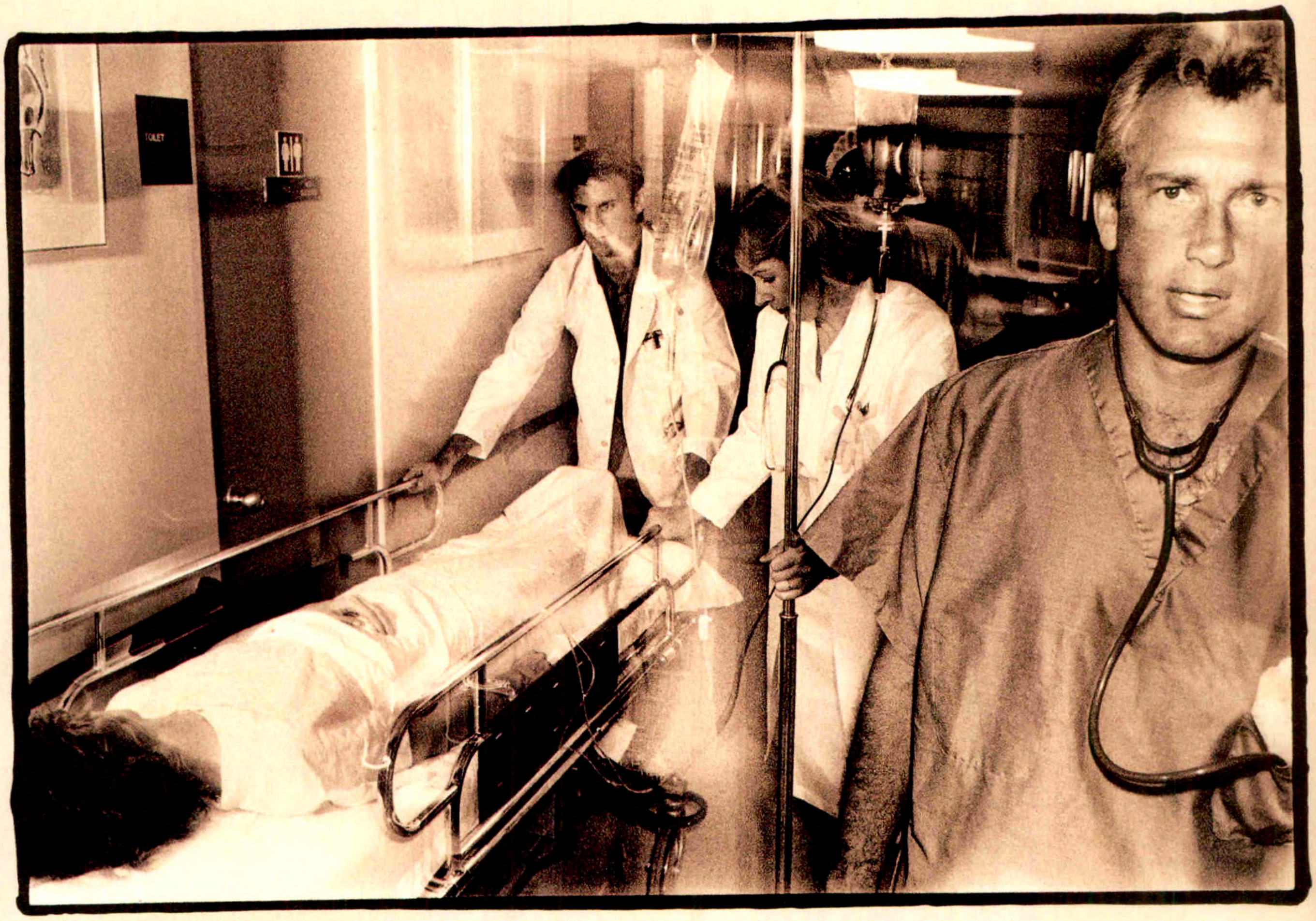

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PRECAUTIONS
General: A high percentage of patients with mononucleosis who receive ampicillin develop a skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. In patients treated with UNASYN the possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.
Drug Interactions: Probenecid decreases the renal tubular secretion of ampicillin and sulbactam. Concurrent use of probenecid with UNASYN may result in increased and prolonged blood levels of ampicillin and sulbactam. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with UNASYN and allopurinol administered concurrently. UNASYN and aminoglycosides should not be reconstituted together due to the *in vitro* inactivation of aminoglycosides by the ampicillin component of UNASYN.
Drug/Laboratory Test Interactions: Administration of UNASYN will result in high urine concentrations of ampicillin. High urine concentrations of ampicillin may result in false positive reactions when testing for the presence of glucose in urine using Clinitest™, Benedict's Solution or Fehling's Solution. It is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix™ or Testape™) be used. Following administration of ampicillin to pregnant women, a transient decrease in plasma concentration of total conjugated estriol, estriolglucuronide, conjugated estrone, and estradiol has been noted. This effect may also occur with UNASYN.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been performed to evaluate carcinogenic or mutagenic potential.
Pregnancy
Pregnancy Category B: Reproduction studies have been performed in mice, rats, and rabbits at doses up to ten (10) times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to UNASYN. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed. (See—Drug/Laboratory Test Interactions.)
Labor and Delivery: Studies in guinea pigs have shown that intravenous administration of ampicillin decreased the uterine tone, frequency of contractions, height of contractions, and duration of contractions. However, it is not known whether the use of UNASYN in humans during labor or delivery has immediate or delayed adverse effects on the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obstetrical intervention or resuscitation of the newborn will be necessary.
Nursing Mothers: Low concentrations of ampicillin and sulbactam are excreted in the milk; therefore, caution should be exercised when UNASYN is administered to a nursing woman.
Pediatric Use: The efficacy and safety of UNASYN have not been established in infants and children under the age of 12.
ADVERSE REACTIONS
UNASYN is generally well tolerated. The following adverse reactions have been reported.
Local Adverse Reactions
Pain at IM injection site—16%
Pain at IV injection site—3%
Thrombophlebitis—3%
Systemic Adverse Reactions
The most frequently reported adverse reactions were diarrhea in 3% of the patients and rash in less than 2% of the patients.
Additional systemic reactions reported in less than 1% of the patients were itching, nausea, vomiting, candidiasis, fatigue, malaise, headache, chest pain, flatulence, abdominal distension, glossitis, urine retention, dysuria, edema, facial swelling, erythema, chills, tightness in throat, substernal pain, epistaxis and mucosal bleeding.
Adverse Laboratory Changes
Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:
Hepatic: Increased AST (SGOT), ALT (SGPT), alkaline phosphatase, and LDH.
Hematologic: Decreased hemoglobin, hematocrit, RBC, WBC, neutrophils, lymphocytes, platelets and increased lymphocytes, monocytes, basophils, eosinophils, and platelets.
Blood Chemistry: Decreased serum albumin and total proteins.
Renal: Increased BUN and creatinine.
Urinalysis: Presence of RBC's and hyaline casts in urine.
The following adverse reactions have been reported with ampicillin-class antibiotics and can also occur with UNASYN.
Gastrointestinal: Gastritis, stomatitis, black "hairy" tongue, enterocolitis and pseudomembranous colitis.
Hypersensitivity Reactions: Urticaria, erythema multiforme, and an occasional case of exfoliative dermatitis have been reported. These reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, the drug should be discontinued, unless the opinion of the physician dictates otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with a penicillin (see WARNINGS).
Hematologic: In addition to the adverse laboratory changes listed above for UNASYN, agranulocytosis has been reported during therapy with penicillins. All of these reactions are usually reversible on discontinuation of therapy and are believed to be hypersensitivity phenomena.

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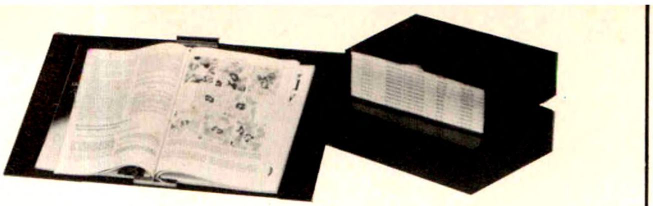

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SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN, INTRAVENOUS STEROIDS, AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.

PRECAUTIONS

General: A high percentage of patients with mononucleosis who receive ampicillin develop a skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. In patients treated with UNASYN the possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.

Drug Interactions: Probenecid decreases the renal tubular secretion of ampicillin and sulbactam. Concurrent use of probenecid with UNASYN may result in increased and prolonged blood levels of ampicillin and sulbactam. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with UNASYN and allopurinol administered concurrently. UNASYN and aminoglycosides should not be reconstituted together due to the *in vitro* inactivation of aminoglycosides by the ampicillin component of UNASYN.

Drug/Laboratory Test Interactions: Administration of UNASYN will result in high urine concentrations of ampicillin. High urine concentrations of ampicillin may result in false positive reactions when testing for the presence of glucose in urine using Clinitest™, Benedict's Solution or Fehling's Solution. It is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix™ or Testape™) be used. Following administration of ampicillin to pregnant women, a transient decrease in plasma concentration of total conjugated estriol, estriolglucuronide, conjugated estrone, and estradiol has been noted. This effect may also occur with UNASYN.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been performed to evaluate carcinogenic or mutagenic potential.

Pregnancy

Pregnancy Category B: Reproduction studies have been performed in mice, rats, and rabbits at doses up to ten (10) times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to UNASYN. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed. (See—Drug/Laboratory Test Interactions.)

Labor and Delivery: Studies in guinea pigs have shown that intravenous administration of ampicillin decreased the uterine tone, frequency of contractions, height of contractions, and duration of contractions. However, it is not known whether the use of UNASYN in humans during labor or delivery has immediate or delayed adverse effects on the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obstetrical intervention or resuscitation of the newborn will be necessary.

Nursing Mothers: Low concentrations of ampicillin and sulbactam are excreted in the milk; therefore, caution should be exercised when UNASYN is administered to a nursing woman.

Pediatric Use: The efficacy and safety of UNASYN have not been established in infants and children under the age of 12.

ADVERSE REACTIONS

UNASYN is generally well tolerated. The following adverse reactions have been reported.

Local Adverse Reactions

Pain at IM injection site—16% Pain at IV injection site—3%
Thrombophlebitis—3%

Systemic Adverse Reactions

The most frequently reported adverse reactions were diarrhea in 3% of the patients and rash in less than 2% of the patients.

Additional systemic reactions reported in less than 1% of the patients were itching, nausea, vomiting, candidiasis, fatigue, malaise, headache, chest pain, flatulence, abdominal distension, glossitis, urine retention, dysuria, edema, facial swelling, erythema, chills, tightness in throat, substernal pain, epistaxis and mucosal bleeding.

Adverse Laboratory Changes

Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:

Hepatic: Increased AST (SGOT), ALT (SGPT), alkaline phosphatase, and LDH.
Hematologic: Decreased hemoglobin, hematocrit, RBC, WBC, neutrophils, lymphocytes, platelets and increased lymphocytes, monocytes, basophils, eosinophils, and platelets.
Blood Chemistry: Decreased serum albumin and total proteins.
Renal: Increased BUN and creatinine.
Urinalysis: Presence of RBC's and hyaline casts in urine.

The following adverse reactions have been reported with ampicillin-class antibiotics and can also occur with UNASYN.

Gastrointestinal: Gastritis, stomatitis, black "hairy" tongue, enterocolitis and pseudomembranous colitis.

Hypersensitivity Reactions: Urticaria, erythema multiforme, and an occasional case of exfoliative dermatitis have been reported. These reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, the drug should be discontinued, unless the opinion of the physician dictates otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with a penicillin (see WARNINGS).

Hematologic: In addition to the adverse laboratory changes listed above for UNASYN, agranulocytosis has been reported during therapy with penicillins. All of these reactions are usually reversible on discontinuation of therapy and are believed to be hypersensitivity phenomena.

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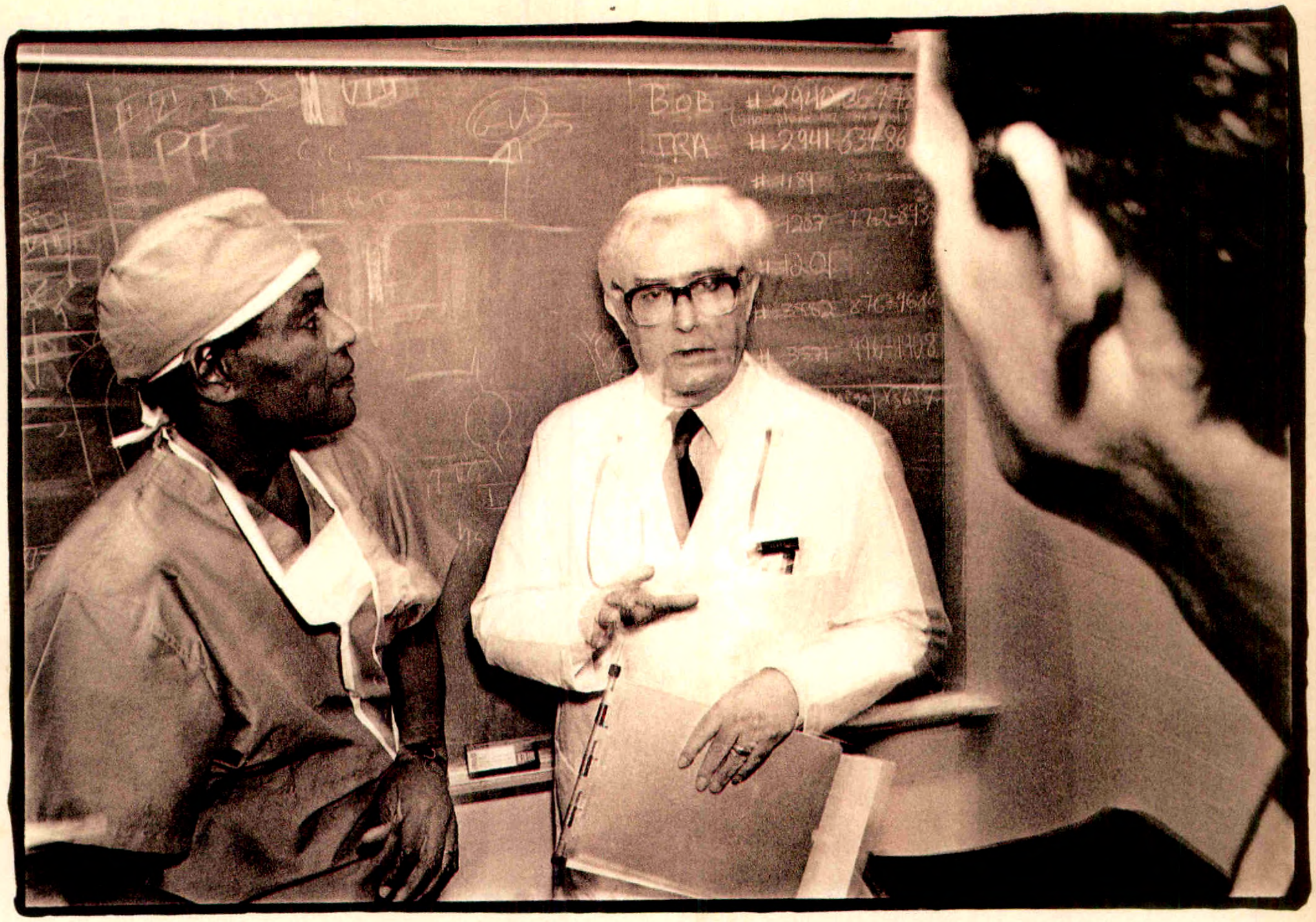

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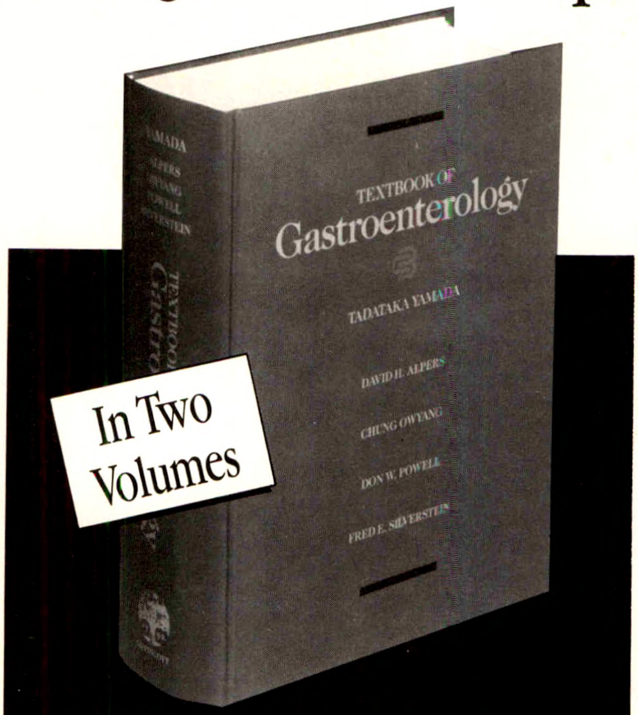

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Acute Appendicitis Following Blunt Abdominal Trauma

Incidence or Coincidence?

MARK H. HENNINGTON, M.D., ELLIS A. TINSLEY, JR., M.D., HERBERT J. PROCTOR, M.D.,*
and CHRISTOPHER C. BAKER, M.D.

From The Division of Trauma, Department of Surgery, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Inflammation of the appendix is a common cause of acute abdominal pain. The etiology and pathophysiology of appendicitis have been well described. The initiating factor often is obstruction of the appendiceal lumen by inspissated stool, barium, food, parasites, or hyperplastic lymphoid tissue. Two patients have been identified who developed appendicitis temporally related to blunt abdominal trauma, without other clear etiology. Although absolute documentation of trauma as an etiologic factor in these cases is difficult, theoretical mechanisms for the occurrence are discussed. In the setting of right lower quadrant pain following mild to moderate blunt abdominal trauma, acute appendicitis should be considered as a possibility.

ACUTE APPENDICITIS IS the most common acute surgical condition of the abdomen. Primary bacterial invasion of the appendiceal lymphoid tissue described by Aschoff[1] dominated theories as to the etiology of the disease until the late 1930s. This 'catarrhal appendicitis' was believed to be due to increased virulence of enteric bacteria or to regional enteritis. The concept of obstruction as the cause had been described as early as 1896 in separate work by Dieulafoy[2] and Talamon[3]; however it was not until the classic work of Wangensteen[4] in 1937 that obstruction was established as the principal cause of acute appendicitis. He and his colleagues[5-7] reproduced the disease in rabbits, apes, and humans by ligation of the appendix. Inflammation occurred despite eradication of resident flora by irrigation. Recently Pieper[8] duplicated these results in rabbits and showed that the length and degree of obstruction correlate with the severity of the disease. Various mechanisms of obstruction have been reported. Trauma as a cause of obstruction resulting in appendicitis has only been alluded to anecdotally.[9-12] We present two cases of appendicitis in patients who presented to the emergency department with abdominal pain following mild to moderate blunt trauma to the lower abdomen.

Case 1

A 46-year-old auto mechanic presented to the emergency department complaining of abdominal pain. He related the onset of pain to an incident that occurred 2 days earlier: a V8 engine transmission weighing at least 200 pounds fell on his lower abdomen and pelvis. The initial pain was 'soreness and stiffness,' which resolved. Then he experienced a 'dull aching all the way across' the lower abdomen, radiating into the back and groin. He also complained of hesitancy, straining to void, and difficulty 'catching my breath.' He was able to eat in small amounts but his appetite had been poor. He was nauseated, but had not vomited and had a normal bowel movement the night before admission. He denied fever, chills, or other symptoms of pain before the accident.

Physical examination revealed that he was in mild respiratory distress, with temperature of 38.6 C, pulse of 88, respirations of 30, and blood pressure of 150/100. Examination was unremarkable except for the abdomen, which was distended with some low-pitched bowel sounds. No tenderness on movement or no peritoneal irritation were noted. There was bilateral lower quadrant pain to palpation that was worse on the right and referred to the suprapubic region. No hernias or masses were palpated. Rectal tone was normal, with a firm prostate and soft, hematest-negative stool. Urinalysis revealed ketones, moderate white blood cells without bacteria, and a small number of red blood cells. Complete blood count revealed a leukocytosis of 12,900 with a left shift and hemoglobin of 14. Plain films were unremarkable. A cystogram revealed no evidence of extravasation. A computed tomographic scan showed a 'small amount of fluid (? blood) in the region of the cecum and mesentery' without retroperitoneal injury.

The patient was admitted for observation. Following admission he remained febrile but hemodynamically stable. He became more distended, his pain increased, and his white blood cell count increased to 16,900. Midline celiotomy was performed and a gangrenous appendix

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* Deceased.

Reprints will not be available.

Address correspondence to Christopher C. Baker, M.D., Division of Trauma, Department of Surgery, School of Medicine, University of North Carolina at Chapel Hill, Burnett-Womack Building 229H, Chapel Hill, NC 27514.

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was found. On closer exam, marked edema of the cecum was noted, with several small areas of subserosal hemorrhage. Appendectomy was performed and no fecalith was identified. The patient did well after operation and was discharged 3 days later. The possibility of transection and/or thrombosis of the small blood vessels was entertained.

Case 2

The second patient was a 12-year-old boy who was brought to the emergency department approximately 12 hours after falling off his bicycle. During the accident he had been struck in the lower abdomen by the handle bars of the bicycle. He had no loss of consciousness and the handle bar injury seemed superficial. Eight hours after the accident the child began to experience vague abdominal discomfort followed by mild nausea and anorexia. In the next 4 hours the pain intensified and the child was first brought to the emergency room for evaluation.

On presentation he appeared ill with a temperature of 38.2 C and blood pressure of 110/60. He complained of abdominal pain. Examination revealed several superficial abrasions, but no other sequelae from the accident were visible. The abdomen was quiet, nondistended, and there was diffuse lower quadrant tenderness. On deep palpation no mass was appreciated; however voluntary and involuntary guarding were elicited on deep palpation of the right lower quadrant. Rectal examination was normal. Laboratory results were remarkable for a hematocrit of 43%, a white blood cell count of 13,000, a normal urinalysis, and a normal serum amylase. The child was admitted for observation. During the next 4 hours he continued to be febrile with continued right lower quadrant pain. He was taken to the operating room and found to have acute suppurative appendicitis. Appendectomy was performed and the child was discharged on the second postoperative day.

Discussion

Approximately 7% of individuals in the western hemisphere develop appendicitis during their lifetime. The peak incidence is during the second and third decades of life. Two hundred thousand appendectomies for acute appendicitis are performed each year in the United States. In Africa and Asia there is a lower incidence, which has been attributed to the high-residue diet consumed in these areas.[13]

The natural history of acute appendicitis is inflammation leading to perforation in less than 36 hours. In 60% to 70% of acutely inflamed appendices, obstruction of the proximal lumen by fecaliths, fibrous bands, parasites, or tumors can be demonstrated. The specimen should be examined closely at the time of operation. Hyperplastic lymphoid tissue resulting from viral illness also has been shown to cause obstruction. Lymphoid hyperplasia may be the most common cause of luminal compromise.[14] Regardless of the cause of luminal obstruction, the subsequent series of events leading to appendiceal inflammation is the same.

Van Zwalenburg[15] in 1904 suggested that obstruction leads to distention of the appendiceal lumen as a result of mucosal secretion. The distention increases with continued mucosal secretion and the rapid multiplication of resident bacteria. Anaerobes and other gram-negative organisms produce a variety of proteolytic enzymes. A closed loop is created similar to the intestinal closed loop first described by Whipple[16] in 1912. As pressure in the submucosa increases, venous pressure is exceeded and capillary and venous occlusion result. Arterial inflow continues and causes progressive vascular engorgement and congestion. The appendiceal mucosa is compromised by impairment of its vascular supply allowing proteolysis and bacterial invasion of the wall. This usually occurs in the mid-appendiceal antimesenteric border due to the tenuous endarteriolar status of this region. In the setting of continued ischemia, bacterially induced proteolysis leads to microinfarcts and subsequent perforation.

The association of acute appendicitis with blunt abdominal trauma is unclear. Some have considered an association to be coincidental. Because blunt abdominal trauma and acute appendicitis both occur with some frequency, and largely in the same population, this hypothesis is reasonable. Others have theorized that appendicitis may actually precede the traumatic events with vague symptoms compromising the patient so as to place him or her at greater risk for trauma. In other words, the physician should consider that, for instance, the child may have fallen off his bicycle due to abdominal pain in the first place. Furthermore it is probable that the patient, and therefore the physician, becomes focused on the traumatic event to the exclusion of early symptoms of appendicitis.

Whether blunt abdominal trauma can be the inciting event leading to obstruction and subsequent acute inflammation is difficult to substantiate. It is feasible, however, that trauma could set into motion the same vicious cycle proposed by Van Zwalenburg. Wells[17] found no development of appendicitis in five rabbits following direct crush injury to the mucosa in the absence of obstruction. Dennis[18] found the opposite result in two rabbits. Direct trauma might lead to edema formation, hematoma, and/or hyperplasia of intrinsic lymphoid tissue with subsequent obstruction of the lumen. Indirectly ileocecal hematomas, mesenteric disruption, edema with enlarged mesenteric nodes, or displacement of stool could easily obstruct the lumen. Features contributing to the obstruction and subsequent development of the disease would include the position of the appendix, mesentery and contained stool at the moment of injury, the actual mechanism of injury, the anatomy of the appendix, mesoappendix, and vascular supply, the resident bacterial flora, and the host immune response.

Although a causative relationship between trauma and appendicitis cannot be proved in the two cases presented, the temporal relationship certainly raises questions of causality Clearly appendicitis can be a difficult diagnosis to make in a hospitalized patient, and trauma is no exception to this aphorism. Because appendicitis and blunt trauma are both common entities, their coexistence (even if coincidental) is certainly feasible. Based on our experience with these two cases, we suggest that appendicitis be considered in the patient with right lower quadrant pain and tenderness following blunt trauma.

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Small-volume Hypertonic Saline Dextran Resuscitation from Canine Endotoxin Shock

JURETA W. HORTON, PH.D., and PAULA B. WALKER, B.S.

From the Department of Surgery, The University of Texas Southwestern Medical Center, Dallas, Texas

This study evaluated resuscitation of endotoxin shock with 7.5% hypertonic saline dextran (HSD 2400 mOsm) by measuring hemodynamic and regional blood flow responses. Endotoxin challenge (1 mg/kg) in adult dogs caused a significant decrease in mean arterial blood pressure (MABP), cardiac output (CO), left ventricular ± dP/dt max, and regional blood flow (radioactive microspheres). Cardiocirculatory dysfunction and acid–base derangements persisted throughout the experimental period in untreated endotoxin shock (group 1, n = 10). In contrast both regimens of fluid resuscitation (group 2, n = 11: bolus of 4 mL/kg HSD followed by a constant infusion of lactated Ringer's [LR] to maintain MABP and CO at baseline values; group 3, n = 10; LR alone given as described for group 2) improved regional perfusion and corrected acid–base disturbances similarly in all dogs. Hypertonic saline dextran enhanced all indices of cardiac contraction and relaxation more than LR alone. The total volume of LR required to maintain MABP and CO at baseline values was less in the HSD group (59.2 ± 6.8 mL/kg) than in the LR alone group (158 ± 16 mL/kg, p = 0.01). The net fluid gain (infused volume minus urine output and normalized for kilogram body weight) was five times greater in the LR (24.8 ± 6.2 mL/kg) than in the HSD group (4.6 ± 1.2 mL/kg, p = 0.01). Lung water was similar in all dogs, regardless of the regimen of fluid resuscitation. Hypertonic saline dextran effectively resuscitates endotoxin shock in this canine model.

PREVIOUS STUDIES HAVE shown that endotoxin shock in dogs depresses cardiocirculatory function, and, if unresuscitated, is associated with a significant mortality rate.[1-6] Fluid resuscitation of the septic subject improves survival but the volume of isotonic fluid required to restore cardiocirculatory function is large, often promoting edema. Recent work by us and others has shown that a small volume of sodium chloride (2400 mOsm), 6% dextran 70 effectively resuscitates subjects in hemorrhagic[7-10] or burn shock[11,12] as well in subjects with acute pancreatitis.[12] This resuscitation regimen has been shown to improve cardiac output and mean arterial blood pressure to near baseline values while reducing the total volume requirements of resuscitation. Recently Holcroff and colleagues[13] reported that small-volume hypertonic saline dextran (HSD) resuscitation during helicopter transport of severely injured patients significantly improved blood pressure and survival more than an identical volume of isotonic resuscitation solution, providing evidence that this solution safely and effectively resuscitates injured patients.

In the present study, we evaluated the resuscitation of endotoxin shock with HSD 70 solution by measuring cardiodynamic, metabolic, and hormonal responses as well as regional perfusion of various organs. The study was designed to determine if HSD could effectively reduce the volume of traditional isotonic resuscitation from endotoxin shock.

Materials and Methods

Thirty-one adult male mongrel dogs (weighing between 21 and 35 kg) were housed for several days in individual cages until a complete blood count and negative screen for microfilaria were obtained. All animals were allowed water *ad libitum* but no food starting 20 hours before the experiment. After sedation with intramuscular acepromazine (0.005 mL/kg), a peripheral venous catheter was placed. Intravenous sodium pentobarbital (15 mg/kg) was administered to induce anesthesia. Each dog was intubated and allowed to breathe spontaneously. Anesthesia was maintained with intravenous alpha chloralose (50 mg/kg) in solution with polyethylene glycol 200. Each animal was instrumented as previously described by this laboratory.[14 15] Body temperature was measured with a rectal

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Address reprint requests to Jureta W. Horton, Ph.D., Department of Surgery, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9031.

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probe (Telethermometer, Yellow Springs, Inc., Yellow Springs, OH). Urine was collected in a closed drainage bag and volume determined every 60 minutes with a graduated cylinder.

Both femoral arteries were catheterized for continuous blood pressure recording (Statham Transducer, Model P23ID, Spectromed, Oxnard, CA) and for simultaneous blood withdrawal for measurement of regional blood flow using the microsphere technique. A microtipped pressure-sensitive transducer (no. 7 French, Model PC470, Millar Instruments, Houston, TX) was placed in the left ventricle by retrograde cannulation of the left carotid artery. This catheter was used for injection of microspheres and for continuous measurement of left heart pressures. The right femoral artery catheter was attached to a Gilford syringe pump (Model 105-S, Gilford Laboratories, Oberlin, OH) to allow blood withdrawal through the cardiac output densitometer (Model COR-100, Waters Instruments, Rochester, MN) during injection of 2.5 mg of cardiogreen dye. A balloon-tipped Swan-Ganz catheter (Model 93A-831H-7-5F, American Edwards, Irvine, CA) was introduced into the right jugular vein, threaded through the right heart and into the pulmonary artery under fluoroscopic guidance. Position was subsequently confirmed by waveform monitoring. A catheter in the left jugular vein was advanced under fluoroscopy into the coronary sinus and subsequent catheter position was confirmed at autopsy. Systemic anticoagulation was achieved by administration of intravenous heparin (100 units/kg).

Regional blood flow in the heart, pancreas, kidney, and splanchnic organs was determined by the radioactive microsphere technique. Six radioactive labels (125-I, 95-Nb, 46-Sc, 85-Sr, 57-Co, and 113-Sn) measuring 10 μ in diameter were used. For each measurement of flow, a bolus of labeled microspheres (approximately 1.5 million) was injected into the left ventricle and the catheter flushed with 10 mL of warm saline. Simultaneous with the injection of microspheres and continuing for 120 seconds, duplicate reference blood samples were obtained from the femoral arteries, each at a constant withdrawal rate of 12 mL/minute (Holter Pump, Model 911, Critikon Laboratories, Tampa, FL). After completing each experiment, an overdose of barbiturates was administered to the dog. The heart, right and left kidneys, and all splanchnic organs were removed. After fixation, the heart was divided into anterior and posterior septum and anterior and posterior left ventricular free wall segments. Each section of left ventricle was then divided further into endocardial and epicardial regions of equal thickness. At least 100 g of each organ was collected for blood flow measurement. Blood and tissue samples were weighed to the nearest milligram and counted for 5 minutes in a multichannel autogamma scintillation spectrometer (Model 5320, Packard Inst., Legona Hills, CA). For each set of samples, vials containing aliquots of each microsphere were used to determine the energy level setting on the scintillation counter. From the standards of each microsphere used to set the energy level and the blood samples containing the highest count of microspheres injected, the cross-talk fraction or overlap of gamma energy was calculated for each of the microspheres. The contribution of overlap of gamma energy of each microsphere was subtracted and a nuclide reference factor (mL/min/count) was calculated. For each tissue sample, the background counts were subtracted and the counts for each isotope were multiplied by the nuclide reference factor to obtain mean blood flow rate. Only animals with less than 15% disparity between right and left kidney flows were used for data analysis. Flows were calculated using a DEC-10 (Digital Equipment Co., Maynard, MA) computer program and expressed as mL/min/g.

Left ventricular pressure, systemic blood pressures, and pulmonary artery pressures were measured continuously. Heart rate was calculated from the left ventricular pressure tracing and recorded at a paper speed of 100 mm/second. Maximal rate of left ventricular pressure increase (+dP/dt max) was obtained with a derivative computer (model 8814A, Hewlett-Packard, Richardson, TX). The dP/dt at a developed pressure of 40 mmHg (dP/dt-DP40), a parameter that is affected minimally by changes in preload and afterload, was calculated as previously described.[16] Total peripheral resistance, pulmonary vascular resistance, and stroke work were calculated as previously described by this laboratory.[15]

Pulmonary and peripheral artery as well as coronary sinus blood samples were withdrawn before the injection of endotoxin, 30 minutes after endotoxin challenge, and hourly after initiating fluid resuscitation. Blood gases, pH, and oxygen content were measured (model ABL 300, Radiometer Blood Gas Analyzer, Radiometer American, Inc., Dallas, TX). Enzymatic methods were used to determine concentrations of lactate in arterial and coronary sinus blood (Sigma Chemical Co., St. Louis, MO). Oxygen delivery, extraction, and consumption by the myocardium were calculated as previously described by this laboratory.[15] Serum sodium, potassium, and chloride levels were measured before endotoxin injection and hourly thereafter (Nova Model 5, Electrolyte Analyzer, Nova Biochemical, Waltham, MA).

At each sampling period, blood was collected from the arterial catheter and assayed for plasma glucose concentration (Semiautomated analyzer). Serum insulin, somatostatin, and glucagon concentrations were measured by radioimmunoassay courtesy of Dr. Roger Unger's laboratory (VA Medical Center, Dallas, TX).[17,18]

Immediately after instrumentation, all baseline hemodynamic and metabolic parameters were measured. A microsphere was injected to measure regional blood flow.

The animals then were given an injection of endotoxin (1 mg/kg body weight); 30 minutes after endotoxin challenge, all parameters were measured again and the animals were divided randomly into three groups. In group 1 (10 dogs), no fluid resuscitation was initiated and these animals served as the untreated endotoxin group. In group 2 (11 dogs), each dog was given intravenously 4 mL/kg body weight HSD (2400 mOsm sodium chloride, 6% dextran 70; Macrodex, Pharmacia, Piscataway, NJ). Lactated Ringer's (LR) solution was infused during a period of 4 hours as required to maintain mean arterial blood pressure and cardiac output at baseline values. In group 3 (10 dogs), endotoxin was injected as described for group 1. Resuscitation consisted of LR solution, infused to maintain mean arterial pressure and cardiac output as described for group 2. After 4 hours of fluid resuscitation, animals were killed with an overdose of barbiturates according to the guidelines set by the National Institutes of Health and The University of Texas Southwestern Medical School Committee for Animal Use. At autopsy the gross appearance of the organs in the abdominal and thoracic cavity was noted and tissues for dry/wet weight ratios and for blood flow studies were collected. Intrathoracic catheter placement was verified.

All data are presented as mean ± standard error of the mean (SEM). For data with multiple points in the experiment, an analysis of variance and a repeated measures procedure (Newman-Keuls) were used to evaluate significant differences between groups with regard to hemodynamic and metabolic parameters as well as changes in regional blood flow. Differences were considered significant at $p < 0.05$.

Results

One of the ten untreated dogs died 65 minutes after endotoxin injection (10% mortality rate); in contrast all of the treated dogs survived the entire experimental period, regardless of the regimen of fluid resuscitation. There was no significant difference in the immediate response to endotoxin challenge in the three experimental groups. Thirty minutes after endotoxin injection, mean arterial blood pressure had decreased similarly in all groups (−42%); a significant decrease in cardiac output and stroke volume (−34% and −32%, respectively) was accompanied by no change in heart rate and peripheral vascular resistance (Table 1). Impaired left ventricular function after untreated endotoxin challenge was indicated by a progressive decrease in the rate of left ventricular pressure rise (+dP/dt max) and fall (−dP/dt max) as well as dP/dt at a developed pressure of 40 mmHg (Fig. 1). In contrast both regimens of fluid resuscitation from endotoxin shock increased ±dP/dt max above baseline values; however 3 and 4 hours after initiating resuscitation, ±dP/dt max and

TABLE 1. *Hemodynamic Response to Endotoxin Challenge*

	Cardiac Output (L/min)			Heart Rate (beats/min)			Stroke Volume (mL)			Peripheral Vascular Resistance (units)		
Groups	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR
Control	3.31 ± 0.19	3.44 ± 0.28	3.50 ± 0.29	86 ± 6	88 ± 5	81 ± 4	38.02 ± 2.72	39.03 ± 3.69	41.80 ± 3.80	33.77 ± 2.33	33.94 ± 3.89	30.20 ± 2.93
Endotoxin ——————→												
30 minutes	2.18† ± 0.22	2.10† ± 0.19	2.25† ± 0.32	96 ± 8	94 ± 6	84 ± 8	26.00† ± 3.00	22.00† ± 1.38	29.92† ± 5.22	27.83 ± 4.09	29.09 ± 1.92	29.98 ± 3.70
Resuscitation Initiated ——————→												
After resuscitation												
60 minutes	1.48† ± 0.14	3.43* ± 0.28	3.64* ± 0.35	105 ± 9	111 ± 12	96 ± 3	13.73† ± 1.49	33.87* ± 4.15	37.81* ± 3.32	21.87† ± 1.50	18.60† ± 1.13	22.46† ± 2.34
120 minutes	1.38† ± 0.12	2.44* ± 0.26	3.65* ± 0.31	119 ± 7	103 ± 11	97 ± 5	12.28† ± 1.45	34.10* ± 4.05	37.95 ± 3.50	23.65† ± 1.68	24.34† ± 1.58	24.71 ± 2.82
180 minutes	1.66† ± 0.15	3.40* ± 0.30	3.41* ± 0.32	108 ± 4	110 ± 11	86 ± 5	15.66† ± 1.54	31.79* ± 2.79	42.30* ± 4.15	20.02† ± 1.60	24.34† ± 1.58	30.20 ± 4.50
240 minutes	1.48† ± 0.13	3.51* ± 0.34	3.86* ± 0.32	104 ± 5	110 ± 10	96 ± 5	13.64† ± 1.19	37.02* ± 4.37	36.73* ± 3.21	39.37† ± 3.95	24.20 ± 2.14	30.00 ± 3.72

All values are mean ± SEM.

Groups include group ENDO, endotoxin challenge only, no treatment, n = 10; group HSD, endotoxin challenge treated with a bolus of hypertonic saline dextran (HSD) plus lactated Ringer's, n = 11; group LR, endotoxin challenge treated with lactated Ringer's only, n = 10.

* Indicates a significant difference between groups at $p < 0.05$.

† Indicates a significant change from baseline within a group at $p < 0.05$.

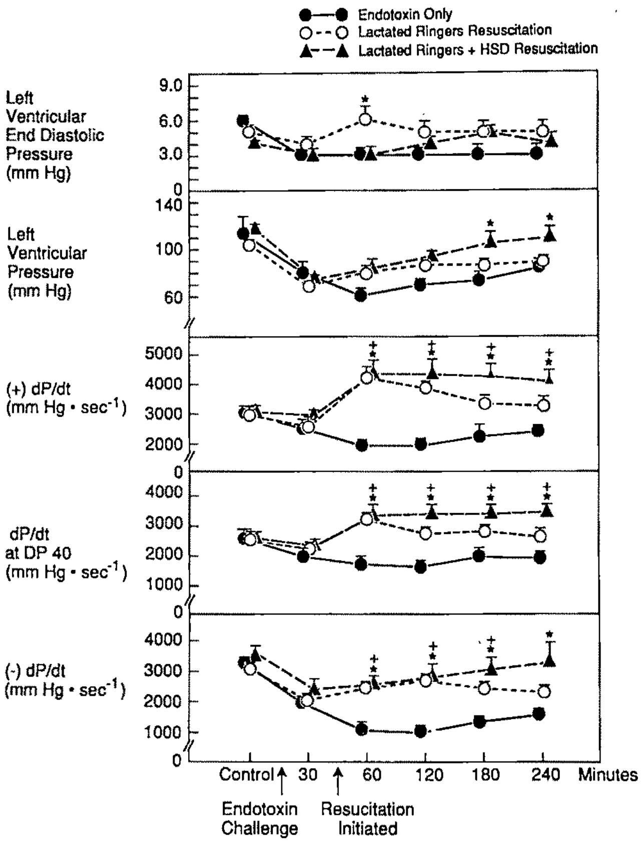

FIG. 1. Cardiodynamic responses to endotoxin challenge and fluid resuscitation. All values are mean ± SEM. *Indicates a significant difference between groups at $p < 0.05$. + Indicates a significant change from baseline at $p < 0.05$.

dP/dt at DP40 were increased to a significantly greater extent in the HSD-treated compared to the LR solution-treated group.

After endotoxin injection, coronary blood flow, myocardial oxygen delivery, endocardial/epicardial flow ratio, and coronary vascular resistance decreased progressively from baseline values in the untreated dogs while myocardial oxygen extraction increased during this time (Fig. 2). The initial decrease in coronary blood flow in response to endotoxin challenge (from 1.58 ± 0.15 to 0.69 ± 0.13 mL/min/g) was followed by progressive increase in coronary perfusion throughout the experimental period; however coronary blood flow remained significantly less than baseline values throughout the experimental period in the untreated group (0.99 ± 0.11 mL/min/g at 3 hours after endotoxin injection). Similarly endocardial/epicardial flow ratio remained less than control values while coronary vascular resistance and myocardial oxygen delivery returned to control values. Both regimens of fluid resuscitation increased coronary blood flow and myocardial oxygen delivery above baseline values, and there was no significant difference in these parameters 3 hours after initiating fluid resuscitation. Fluid resuscitation caused a significant decrease in myocardial oxygen extraction, and by 3 hours after initiating volume replacement, extraction ratio was significantly lower in both treated groups compared to the untreated endotoxin dogs.

Untreated endotoxin challenge produced a significant acidosis, as indicated by a decrease in the arterial blood pH and serum bicarbonate level and an increase in arterial and coronary sinus lactate ($p = 0.01$) (Table 2). Arterial PO_2 was not altered significantly by endotoxin challenge. Arterial PCO_2 decreased significantly after endotoxin injection due to an increase in respiratory rate (from 16 ± 1 to 26 ± 2 breaths per minute). Hematocrit ratio progressively increased after untreated endotoxin shock, while serum protein content, serum sodium, potassium, and chloride concentration remained unchanged (Fig. 3). Resuscitation of endotoxin shock with either LR solution alone or with HSD plus LR solution caused a 35% decrease in plasma total protein concentration, while hematocrit remained unchanged. Serum sodium, potassium, and chloride remained unchanged from baseline values in the endotoxin group resuscitated with LR solution alone. While a moderate increase in serum sodium (from

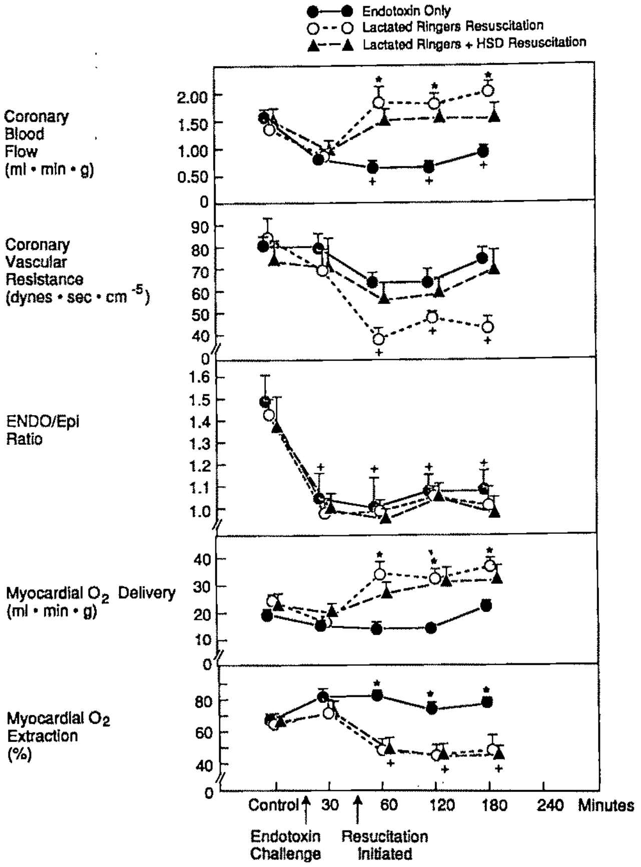

FIG. 2. Coronary blood flow and myocardial oxygen metabolism after endotoxin challenge and fluid resuscitation. All values are mean ± SEM. *Indicates a significant difference between groups at $p < 0.05$. + Indicates a significant change from baseline at $p < 0.05$.

TABLE 2. *Acid–Base Balance After Endotoxin Challenge*

	Arterial pH			Arterial pO2 (mmHg)			Arterial pCO_2 (mmHg)			Arterial HCO_3 (mm/L)			Arterial Lactate (mm/L)		
Groups	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR
Control	7.33 ± 0.02	7.34 ± 0.01	7.32 ± 0.01	99.2 ± 5.4	89.6 ± 4.4	99.6 ± 7.6	38.9 ± 1.7	35.6 ± 1.9	37.4 ± 1.3	20.1 ± 0.3	19.1 ± 0.4	18.8 ± 0.1	0.747 ± 0.094	0.848 ± 0.232	0.841 ± 0.167
Endotoxin →															
30 minutes	7.29† ± 0.01	7.31 ± 0.02	7.28† ± 0.01	104.9 ± 5.2	93.5 ± 9.6	95.0 ± 8.1	31.7† ± 2.1	25.1† ± 1.3	30.7† ± 0.8	14.7† ± 0.8	12.4† ± 0.7	14.5† ± 0.6	2.190 ± 0.116	2.625 ± 0.587	2.719 ± 0.486
Resuscitation Initiated →															
After resuscitation															
60 minutes	7.25† ± 0.03	7.27† ± 0.03	7.28† ± 0.01	107.5 ± 9.5	93.8 ± 8.9	104.3 ± 7.2	30.5† ± 2.5	28.0† ± 1.4	23.9† ± 0.8	12.8† ± 0.5	12.5† ± 0.5	12.0† ± 0.3	2.501 ± 0.181	3.574 ± 0.754	2.770 ± 0.479
120 minutes	7.23† ± 0.02	7.27† ± 0.04	7.29† ± 0.01	109.7 ± 6.5	86.8 ± 8.4	101.2 ± 6.3	26.4† ± 1.3	28.2† ± 1.9	27.4† ± 1.3	10.8† ± 0.6	13.2† ± 0.4	12.7† ± 0.5	2.842 ± 0.196	3.147 ± 0.674	2.291 ± 0.442
180 minutes	7.22† ± 0.03	7.31* ± 0.03	7.29* ± 0.02	108.7 ± 5.9	88.3 ± 6.6	103.1 ± 6.7	28.3† ± 1.6	26.7† ± 1.8	26.6† ± 0.6	11.1† ± 0.6	13.0† ± 0.5	12.5† ± 0.4	2.813 ± 0.239	3.255 ± 0.747	2.378 ± 0.529
240 minutes	7.21† ± 0.04	7.33* ± 0.02	7.33* ± 0.02	97.6 ± 7.1	86.1 ± 6.4	96.5 ± 7.1	25.4† ± 2.5	25.3† ± 1.5	23.9† ± 0.4	11.1† ± 1.0	12.9† ± 0.3	12.3† ± 0.4	2.248 ± 0.268	3.139 ± 0.366	2.095* ± 0.215

All values are mean ± SEM.

Groups include group ENDO, endotoxin challenge only, no treatment, n = 10; group HSD, endotoxin challenge treated with a bolus of hypertonic saline dextran (HSD) plus lactated Ringer's, n = 11; group LR, endotoxin challenge treated with lactated Ringer's only, n = 10.

* Indicates a significant difference between groups at $p < 0.05$.

† Indicates a significant change from baseline within a group at $p < 0.05$.

139 ± 1 to 150 ± 1 mEq/L, p = 0.05) occurred in those dogs given HSD plus LR solution, serum potassium and chloride remained at baseline values. Acidosis persisted transiently after fluid resuscitation from endotoxin challenge; however 4 hours after initiating fluid resuscitation, arterial pH returned to baseline values in all treated dogs. Hypocapnia persisted in all dogs after fluid resuscitation due to sustained increases in respiratory rate. Arterial and coronary sinus lactate levels remained above baseline values throughout the experimental period in all dogs. However 4 hours after initiating fluid resuscitation, arterial lactate was significantly lower in the HSD compared to the LR group (p = 0.05).

Peripheral blood flow (pancreas, spleen, stomach, small bowel, colon, renal blood flow, and non-nutritive lung blood flow) decreased similarly in all dogs immediately after endotoxin challenge. Both regimens of fluid resuscitation improved regional blood flow, and 4 hours after initiating fluid resuscitation there was no significant difference in any measured regional blood flow parameter (Tables 3 and 4).

Endotoxin challenge produced diarrhea in all dogs and this was unchanged by either regimen of fluid resuscitation. Urine output decreased significantly in all dogs after endotoxin injection and urine output remained less than 10 mL/hr in the untreated dogs. Urine flow increased after resuscitation with both regimens of fluid resuscitation, but the 97% increase in flow in the LR group exceeded the 40% increase measured in the HSD group. In the 4.5 hours after administration of endotoxin, the total urine output was 408 ± 48 mL in the LR group and 318 ± 20 mL ($p < 0.05$) in the HSD group. Renal blood flow decreased significantly and progressively throughout the experimental period in untreated endotoxin shock; in contrast renal blood increased to near-baseline values in the dogs given volume replacement, regardless of the regimen of fluid resuscitation. The total volume of LR required to maintain mean arterial blood pressure and cardiac output at baseline values was 158 ± 16 mL/kg in those dogs given LR alone, and 59.2 ± 6.8 mL/kg (p = 0.001) in the HSD group. The net fluid gain, calculated as the infused volume minus urine output and normalized for kilogram body weight, was five times greater in the LR group (24.8 ± 6.2 mL/kg) than in the HSD group (4.6 ± 1.2 mL/kg, p = 0.01) (Fig. 4). Despite the significantly larger volume of crystalloid infused and the greater net fluid gain in the endotoxin dogs treated with LR alone, lung water, calculated by the gravimetric method, was similar in all dogs, regardless of the regimen of fluid resuscitation.

The hormonal response to endotoxin challenge and fluid resuscitation from endotoxin shock are summarized in Table 5. Baseline plasma glucose, insulin, glucagon, and somatostatin were similar in all dogs. Thirty minutes

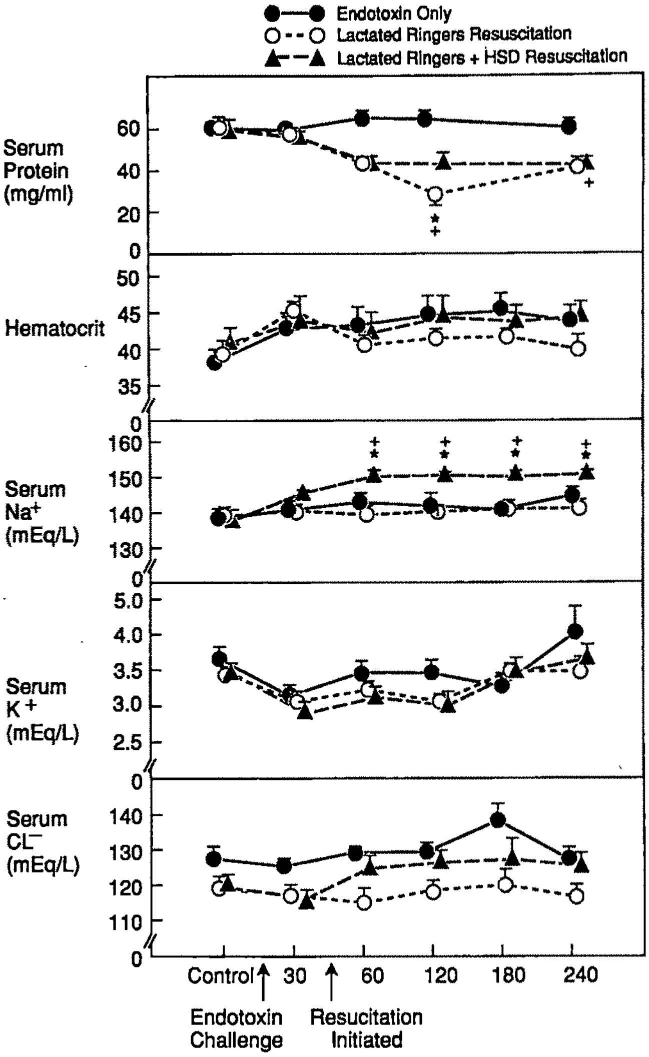

FIG. 3. Endotoxin-mediated changes in serum protein, hematocrit, and electrolyte concentration. Na+, sodium; K+, potassium; CL, chloride. All values are mean ± SEM. *Indicates a significant difference between groups at $p < 0.05$. + Indicates a significant change from baseline at $p < 0.05$.

after endotoxin challenge, plasma glucose, insulin, and glucagon increased significantly (13%, $p = 0.05$; 57%, $p = 0.01$; and 80%, $p = 0.01$, respectively) and somatostatin was unchanged. Four hours after initiating fluid resuscitation from shock, plasma glucose and insulin levels had decreased significantly (−43% and −60% from baseline values, $p = 0.05$, respectively), while plasma glucagon increased 420% during this time ($p = 0.01$). These changes in circulating hormones occurred regardless of whether HSD or LR solution was used to resuscitate endotoxin shock. Four hours after initiating fluid resuscitation, somatostatin decreased 42% from baseline values in those dogs given LR solution alone but was unchanged from baseline values in the HSD-treated groups.

Discussion

Previously we showed that initiating volume replacement with a small volume of HSD reduced the total volume requirements for resuscitation of pancreatitis[12] and burn injury[11] and significantly enhanced cardiac contractile function in both experimental models.[11,12] Similarly HSD (2400 mOsm) has been used successfully in resuscitation of experimental hemorrhagic shock,[7-10,19-22] clinical trauma,[13,23] as well as after operation in coronary bypass patients.[24] Other hypertonic solutions have been used to resuscitate endotoxin shock[25] and clinical burns in adults[26] and children.[27] While reversal of shock occurs rapidly with hypertonic saline resuscitation, the mechanisms underlying improved cardiac contractile function remain speculative.

Data from the present study are consistent with previous reports of hemodynamic and metabolic derangements after endotoxin challenge.[2-6] Cardiocirculatory dysfunction after endotoxin injection included hypotension, reduced preload, decreases in cardiac output and stroke volume and ±dP/dt; in addition redistribution of regional blood flow and profound metabolic acidosis occurred. Lactated Ringer's solution alone and HSD plus LR solution were equally effective in restoring cardiac output and regional perfusion. While fluid resuscitation restored cardiac function in all dogs, all indices of left ventricular contraction–relaxation (systolic left ventricular pressure, ±dP/dt max and dP/dt at DP40) were significantly better 4.5 hours after initiating fluid resuscitation with HSD. The dP/dt at a developed pressure of 40 mmHg was calculated in this study to provide a suitable index of left ventricular contractility; previous studies have shown that this parameter minimizes the modulating effects of preload and after load.[16] Hypertonic-mediated enhancement of left ventricular performance is consistent with a previous report by our laboratory showing that hypertonic resuscitation restored burn-induced deficits in cardiac contraction and relaxation[11,28]; the enhanced cardiac performance was confirmed using an isolated denervated heart model in an environment free of neurohumoral constraints, suggesting a direct effect of hyperosmotic agents on cardiac muscle. In this study the positive inotropic effects of HSD resuscitation probably were related to cellular dehydration and a significant increase in intracellular sodium concentration. The enhanced cellular sodium loading could promote sodium calcium exchange, increasing calcium ion availability to the contractile proteins.[29] These data support a report by Goethals and colleagues[30] attributing enhanced left ventricular performance with hyperosmotic resuscitation to increased intracellular calcium availability and confirm earlier reports by Wildenthal and associates[31] that hyperosmotic solutions enhance cardiac contractility. The improved left

TABLE 3. *Regional Blood Flow (mL/min/g)*

	Liver			Spleen			Stomach			Proximal Bowel			Colon		
Groups	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR
Control	0.31 ± 0.09	0.30 ± 0.07	0.21 ± 0.05	0.82 ± 0.31	1.07 ± 0.38	1.25 ± 0.36	0.37 ± 0.07	0.41 ± 0.12	0.55 ± 0.09	0.70 ± 0.15	1.08 ± 0.22	0.89 ± 0.17	0.77 ± 0.20	0.88 ± 0.15	0.83 ± 0.13
Endotoxin →															
30 minutes	0.47 ± 0.06	0.43 ± 0.09	0.57 ± 0.11	0.71 ± 0.17	0.70 ± 0.16	0.80 ± 0.26	0.20† ± 0.04	0.15† ± 0.03	0.18† ± 0.03	0.61 ± 0.06	0.63 ± 0.08	0.52 ± 0.08	0.55 ± 0.08	0.50† ± 0.11	0.45† ± 0.10
Resuscitation Initiated →															
After resuscitation															
60 minutes	0.32* ± 0.08	0.96 ± 0.18	0.85 ± 0.21	0.60* ± 0.13	1.32 ± 0.15	1.95 ± 0.23	0.14† ± 0.02	0.28 ± 0.05	0.26 ± 0.84	0.28† ± 0.06	0.60 ± 0.11	0.58 ± 0.08	0.32*† ± 0.07	0.89 ± 0.20	0.88 ± 0.18
120 minutes	0.26* ± 0.04	0.80 ± 0.16	1.14 ± 0.27	0.93 ± 0.22	0.87 ± 0.19	1.71 ± 0.28	0.24† ± 0.04	0.33 ± 0.07	0.29 ± 0.04	0.29† ± 0.10	0.55 ± 0.08	0.62 ± 0.08	0.48*† ± 0.12	0.60† ± 0.09	0.83 ± 0.22
240 minutes	0.52* ± 0.11	1.39 ± 0.31	1.07 ± 0.17	0.50† ± 0.10	0.65 ± 0.24	0.79 ± 0.20	0.18† ± 0.03	0.39 ± 0.06	0.31 ± 0.07	0.29† ± 0.02	0.77 ± 0.19	0.59 ± 0.09	0.46*† ± 0.05	0.47*† ± 0.12	0.86 ± 0.21

All values are mean ± SEM.

Groups include group ENDO, endotoxin challenge only, no treatment, n = 10; group HSD, endotoxin challenge treated with a bolus of hypertonic saline dextran (HSD) plus lactated Ringer's, n = 11; group LR, endotoxin challenge treated with lactated Ringer's only, n = 10.

* Indicates a significant difference between groups at $p < 0.05$.

† Indicates a significant change from baseline within a group at $p < 0.05$.

ventricular relaxation seen in this study may be related to the hypertonic-induced increase in the time to peak tension as well as increased duration of contraction and relaxation.[27] As proposed by Beyers and colleagues,[29] prolongation of the duration of relaxation promotes more complete resequestration of calcium in subcellular organelles, such as the sarcoplasmic reticulum, producing the enhanced rate of left ventricular full seen in this present study. In addition more complete resequestration of calcium in the sarcoplasmic reticulum (SR) probably increases calcium availability for release during the subsequent depolarization of SR membranes, contributing to

TABLE 4. *Regional Blood Flow (mL/min/g)*

	Pancreas			Diaphram			Lung			Kidney			Adrenal Gland		
Groups	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR
Control	0.63 ± 0.07	0.54 ± 0.07	0.72 ± 0.12	0.21 ± 0.01	0.25 ± 0.02	0.23 ± 0.05	1.64 ± 0.22	1.92 ± 0.25	1.70 ± 0.21	5.83 ± 0.61	5.94 ± 0.54	5.71 ± 0.48	2.60 ± 0.06	2.75 ± 0.47	2.55 ± 0.08
Endotoxin →															
30 minutes	0.19† ± 0.02	0.20† ± 0.02	0.36† ± 0.05	0.16† ± 0.03	0.26 ± 0.09	0.27 ± 0.05	0.80† ± 0.29	0.72† ± 0.53	0.65† ± 0.17	4.78 ± 0.90	4.99 ± 0.70	4.25 ± 0.45	2.74 ± 0.24	2.82 ± 0.34	2.70 ± 0.30
Resuscitation Initiated →															
After resuscitation															
60 minutes	0.12† ± 0.01	0.37† ± 0.07	0.28† ± 0.03	0.11† ± 0.02	0.39 ± 0.10	0.27 ± 0.03	0.40† ± 0.15	1.75 ± 0.32	1.17 ± 0.26	2.85† ± 0.43	5.64 ± 0.56	5.39 ± 0.80	0.94† ± 0.18	4.20 ± 0.48	3.71 ± 0.55
120 minutes	0.24† ± 0.07	0.25† ± 0.03	0.26† ± 0.04	0.16 ± 0.03	0.26 ± 0.04	0.29 ± 0.06	0.54† ± 0.20	2.41 ± 0.22	0.99† ± 0.14	3.16† ± 0.50	4.86 ± 0.36	5.73 ± 0.63	1.23† ± 0.22	4.37 ± 0.52	4.21 ± 0.45
240 minutes	0.10† ± 0.01	0.24† ± 0.05	0.22† ± 0.03	0.15 ± 0.03	0.38 ± 0.13	0.30 ± 0.05	0.44† ± 0.14	1.33 ± 0.37	1.58 ± 0.15	3.08† ± 0.43	4.54 ± 0.45	4.57 ± 0.59	2.16 ± 0.45	3.24 ± 0.65	3.22 ± 0.26

All values are mean ± SEM.

Groups include group ENDO, endotoxin challenge only, no treatment, n = 10; group HSD, endotoxin challenge treated with a bolus of hypertonic saline dextran (HSD) plus lactated Ringer's, n = 11; group LR, endotoxin challenge treated with lactated Ringer's only, n = 10.

* Indicates a significant difference between groups at $p < 0.05$.

† Indicates a significant change from baseline within a group at $p < 0.05$.

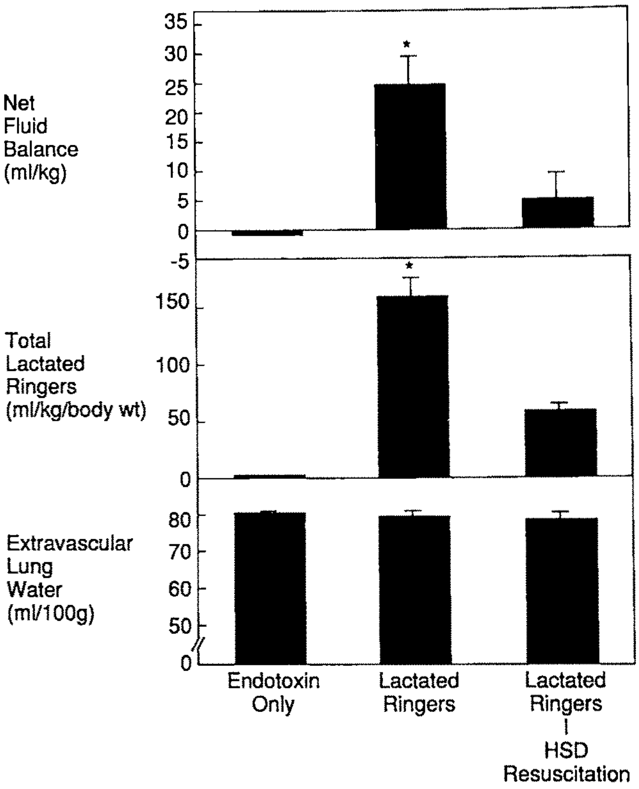

FIG. 4. Net fluid balance, resuscitation volume requirements, and extravascular lung water content after endotoxin challenge. All values are mean ± SEM. *Indicates a significant difference between groups at $p < 0.05$.

the increased rate of left ventricular pressure rise seen in this study.

Another mechanism that may contribute to HSD enhancement of cardiac function is improved myocardial perfusion. In this regard Mazzoni and colleagues showed that HSD increased vessel diameter previously narrowed by an episode of shock, probably due to dehydration of endothelial cells.[32,33] These authors showed that rapid restoration of luminal diameter produced a persistent increase in organ flow. In the present study, we used radioactive microspheres to quantitate total as well as transmural coronary blood flow. While endotoxin challenge reduced total coronary blood flow and significantly impaired subendocardial perfusion, both regimens of fluid resuscitation were equally effective in correcting myocardial perfusion deficits, eliminating HSD-mediated changes in coronary perfusion as the mechanism underlying enhanced inotropic responses to hyperosmotic resuscitation. Our finding of a lower arterial lactate level in the hypertonic saline group accompanied by higher oxygen consumption suggested improved nutrient blood flow to peripheral tissue. These data support previous reports that HSD increases oxygen delivery to peripheral tissue.[19,34,35] However, in our study, both regimens of fluid resuscitation were equally effective in restoring peripheral perfusion, suggesting that increased oxygen delivery to peripheral tissues was not the sole mechanism underlying increased survival described by several investigators after HSD resuscitation from shock.

Previous studies have shown that HSD resuscitation and a subsequent increase in plasma osmolality increase circulating levels of vasopressin and catecholamines.[36] These potent vasoconstrictors would be expected to redistribute cardiac output, favoring some regions of the peripheral circulation while reducing flow in other vascular beds. However both HSD and LR solution were equally effective in correcting endotoxin-mediated renal hypoperfusion, probably due to expansion of the plasma volume and improved venous return. It was of interest that while mean arterial blood pressure, cardiac output, and renal blood flow were nearly identical in all resuscitated dogs, there was a significantly greater increase in urine output in dogs given LR solution alone, probably due to increased free water clearance.[2]

Improved cardiocirculatory function after HSD resuscitation from several types of shock has been attributed to expansion of circulating plasma volume. Kramer and colleagues[7] showed that in adult sheep, 940 to 1260 mL of water could be mobilized from the cellular, extracellular compartment after HSD resuscitation. However, in our study, dogs resuscitated with LR solution alone required 2737 mL more fluid during 4 hours of resuscitation. Despite a significantly lesser volume of LR solution given in the HSD group, serum protein and hematocrit ratio were nearly identical after 4 hours of resuscitation. These data suggest that the enhanced cardiocirculatory function after HSD resuscitation was not related solely to mobilization of water from the intracellular and extracellular compartments.

In this present study, endotoxin challenge produced an immediate hyperglycemia and hyperinsulinemia as well an increase in plasma glucagon, and these responses were probably related to the shock-induced sympathoadrenal activation and an increase in circulating catecholamines. Hypoglycemia and hypoinsulinemia occurred in all dogs throughout fluid resuscitation from endotoxin challenge, supporting previous reports of an endotoxin-mediated increase in glycogen phosphorylase activity and glycogen depletion.[37] Glucagon levels continued to increase throughout the experimental period, and these data are consistent with a previous proposal that glucagon serves as a glycogenolytic agent after endotoxin challenge.[38]

In summary HSD plus LR solution and LR solution alone were equally effective in maintaining perfusion of peripheral tissues and correcting acid–base derangements. Small volume HSD resuscitation of canine endotoxin shock significantly reduced the volume of LR solution required to maintain cardiac output and mean arterial pressure at baseline values. The net fluid gain (resuscita-

TABLE 5. *Hormonal Response to Endotoxin Challenge and Resuscitation*

	Glucose (mg/%)			Insulin (mU/mL)			Glucagon (pg/mL)			Somatostatin (pg/mL)		
Groups	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR	ENDO	LR	HSD/LR
Control	112 ± 4	110 ± 6	116 ± 4	23.1 ± 2.4	24.4 ± 3.8	22.7 ± 3.2	102 ± 10	100 ± 11	105 ± 14	82 ± 6	74 ± 5	67 ± 5
Endotoxin →												
30 minutes	130† ± 7	124† ± 4	126† ± 4	36.3† ± 2.6	36.5† ± 5.5	36.9† ± 6.4	207† ± 77	181† ± 20	188† ± 40	79 ± 5	71 ± 9	75 ± 6
Resuscitation Initiated →												
Post resuscitation												
60 minutes	134 ± 9†	82 ± 9†	87 ± 8†	28.2 ± 6.8	14.6† ± 3.1	23.0 ± 8.0	416*† ± 103	352† ± 89	233† ± 46	180*† ± 8	71 ± 9	78 ± 12
120 minutes	115 ± 11	67† ± 8	70† ± 7	41.2*† ± 1.7	11.0† ± 3.4	16.0† ± 3.2	566*† ± 55	394† ± 95	372† ± 87	170*† ± 10	63 ± 6	74 ± 18
180 minutes	103 ± 11	65† ± 6	60† ± 9	9.3 ± 2.7	7.8† ± 1.0	16.0† ± 5.9	705*† ± 123	428† ± 91	420† ± 84	140*† ± 5	53† ± 12	71 ± 13
240 minutes	101 ± 8	67† ± 5	72† ± 5	15.6 ± 4.5	8.0† ± 0.9	10.8† ± 2.2	1337*† ± 185	489† ± 91	581† ± 114	137*† ± 9	43† ± 11	68 ± 10

All values are mean ± SEM.

Groups include group ENDO, endotoxin challenge only, no treatment, n = 10; group HSD, endotoxin challenge treated with a bolus of hypertonic saline dextran (HSD) plus lactated Ringer's, n = 11; group LR, endotoxin challenge treated with lactated Ringer's only, n = 10.

* Indicates a significant difference between groups at $p < 0.05$.

† Indicates a significant change from baseline within a group at $p < 0.05$.

tion volume infused minus urine output, normalized for kilogram body weight) was significantly greater in the LR group (24.8 ± 6.2 mL/kg) than that measured in the HSD group (4.6 ± 1.2 mL/kg). Hypertonic saline dextran resuscitation improved all indices of cardiac contraction and relaxation more than LR solution resuscitation alone. We conclude that HSD effectively resuscitates endotoxin shock in this canine model.

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Composition of Fat in Enteral Diets Can Influence Outcome in Experimental Peritonitis

MICHAEL D. PECK, M.D., SC.D.,* CORA K. OGLE, PH.D., and J. WESLEY ALEXANDER, M.D., SC.D.

An animal model of protracted acute peritonitis was used to study the effects on survival of different amounts and types of dietary fat. Guinea pigs (n = 132) were provided with gastrostomies and allowed to recover. Intraperitoneal osmotic pumps were then placed, allowing for effusion of viable bacteria during the next week. Three days after pump implantation, the animals were randomized to receive one of nine diets, differing only in fat content (3.5%, 14%, or 56% of total calories) and fat composition (100% Microlipid® [primarily safflower oil, rich in linoleic acid], 100% MaxEPA® [fish oil containing 35% omega-three fatty acids], or a 50:50 mixture of both oils). At the end of 2 weeks, survival rates were compared. Among all types of fat used, the level of fat did not affect outcome, with survival rates of 13% (6 of 45) at the low level of fat used, 30% (13 of 44) at the medium level, and 26% (11 of 43) at the high level ($p > 0.10$). However fat composition significantly influenced survival across all levels of fat, with a 39% (17 of 44) survival rate in the groups given equal amounts of Microlipid® and MaxEPA®, compared either to 21% (9 of 44) for the 100% Microlipid® groups, or to 9% (4 of 44) for the 100% MaxEPA® groups ($p < 0.05$). A second experiment was performed with 58 animals fed five different mixtures of fats, all at 14% of total calories. Diets were made with fat ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 Microlipid® to MaxEPA®. The survival rate in the 50:50 group was 83% (10 of 12), which is significantly higher than the survival rates with any of the others ($p < 0.05$). It is concluded that the amount of fat in the diet does not strongly influence outcome in this model. However an equal mixture of safflower and fish oils significantly improves survival rates compared to diets made with single-oil preparations.

DIETARY NUTRIENTS CAN have a profound effect on the host response to infection, and manipulation of nutrients during states of stress, such as sepsis, burns, or trauma, may provide a significant therapeutic adjunct. Preliminary studies with a defined formula already have shown benefits in critically ill patients,[1,2] but further work is required in meaningful animal models to establish the optimal dose and combination of nutrients for nutritional support during sepsis.

Fatty acids have the potential to modulate host immune response on many levels. One of the most important areas for interaction involves the modulation of eicosanoid (prostaglandin, thromboxane, and leukotriene) synthesis.[3] Fatty acids of the omega-6 family (as found in vegetable oils, including safflower oil) are precursors of prostanoids of the '2' or dienoic series, *e.g.*, prostaglandin E_2 (PGE_2). On the other hand, fatty acids of the omega-3 family (as found in fish oils) are precursors of prostanoids of the '3,' or trienoic series, *e.g.*, prostaglandin E_3 (PGE_3).

The importance of this biochemical divergence is that dienoic prostanoids generally have more potent biologic effects than their trienoic counterparts. For example PGE_3 is less immunosuppressive than PGE_2.[4]

Changes in dietary fat composition were studied to determine their effect on outcome in an animal model of peritonitis. Specifically the effect of different amounts of safflower and fish oils in the animal diets were investigated, as well as different combinations of the two. The model allows enteral feeding for up to 2 weeks during active bacterial peritonitis in previously gastrostomized guinea pigs.[5] Length of survival and survival rates were the outcome variables studied.

Materials and Methods

Preparation of Animals

For at least 1 week before experimental use, individually caged female Hartley guinea pigs weighing a mean of 390

From the Department of Surgery, University of Cincinnati Medical Center and Shriners Burns Institute, Cincinnati, Ohio

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Address reprint requests to J. Wesley Alexander, M.D., Department of Surgery, University of Cincinnati Medical Center, 231 Bethesda Ave., Cincinnati, OH 45267-0558.

Dr. Peck's present address is University of Miami School of Medicine, Miami, Florida.

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g (range, 276 to 456 g) were acclimated to constant laboratory conditions at 26 C and fed Guinea Pig Pellet Diet (Wayne Feeds Research Division, Libertyville, IL) and water *ad libitum*. After this period of acclimation, guinea pigs were provided with intragastric feeding tubes as previously described.[6,7] The free end of the tube was tunneled subcutaneously around the left hemi-thorax to exit through a stab wound in the upper intrascapular region, later to be connected to the diet infusion system. After operation the animals were returned to their previous laboratory conditions for an additional 7 days and allowed to eat *ad libitum*. This allowed time to regain the 10% body weight loss that occurred with surgery.

After this stabilization the animals underwent a laparotomy under general anesthesia to place a bacteria-filled osmotic pump into the right lower quadrant. The guinea pigs then were placed into individual metabolic cages and allowed to eat and drink *ad libitum*. On day 3 the animals were randomized to experimental diets.

Preparation of Bacteria

For 18 hours before implantation of the pump, cultures of *Escherichia coli* 53104 (courtesy of Dr. Richard Simmons, Pittsburgh, PA) and *Staphylococcus aureus* 502A were each incubated in trypticase soy broth in a 37 C oscillating water bath. The cultures were centrifuged at 2000 rpm for 7 minutes, and the resulting pellets washed three times in 0.9% NaCl. After the final wash, the pellets were diluted serially in sterile saline to achieve a final concentration of 2×10^8 bacteria per milliliter. An equal mixture was then made containing 1.0×10^8 each of the *E. coli* and *S. aureus*.

Preparation of the Osmotic Pumps

Using aseptic techniques, each osmotic pump (Alzet Osmotic Pump®, Model 2 MLI, Alza Co., Palo Alto, CA) was filled with 2 mL of the *E. coli* and *S. aureus* mixture, attached to silicone tubing (100 cm in length), and implanted through a laparotomy under general anesthesia as previously described. The pumps have a flow delivery rate of 0.01090 mL/hr (±standard deviation [SD] 0.00600 mL/hr) and begin actual delivery of 1.645 mL of the bacterial suspension into the peritoneal cavity between 34 and 36 hours after implantation. These pumps are designed to provide continuous delivery of their contents for 7 days.[8]

Experimental Groups

On day 3 after laparotomy, the animals were placed on one of the diets differing in amount and composition of fat. The basic diet composition is shown in Table 1. The diets were prepared so that all animals received continuous isovolemic intragastric pump-controlled tube feedings daily (Holter Pump®, Catalogue #903, Critikon, Bound Rock, NY and Kangaroo 330® Feeding Pump, donated by Sherwood Medical, Hospital Products Division, St. Louis, MO).

TABLE 1. *Composition of Basic Diet*

Element	Amount
Protein (Promix R.P.D.®*)	15.5 g/L
NaCl (2.5 mEq/mL)	24 mL
KCl (2 mEq/mL)	10 mL
K acetate (2 mEq/mL)	24 mL
K phosphate (3 mmol/L/mL)	10 mL
Ca Gluconate (0.1 g/mL)	50 mL
MgSO4 (4 mEq/mL)	24 mL
MTE5®‡	6 mL
Multi-vitamin concentrate‖	5 mL
Vitamin C	500 mg
Vitamin E	100 mg
Choline chloride	500 mg
Folic acid	2 mg
Tap water to 1 L final volume	
Nutrisource† varied with experiment	

* Navaco Laboratories, Phoenix, AZ.

† SandozNutrition, Minneapolis, MN.

‡ MulTE-PAK-5™, Smith & Nephew, Franklin Park, IL.

Each liter of diet contains: zinc, 6 mg; copper, 2.4 mg; manganese, 0.6 mg; chromium, 24 mcg; selenium, 120 mcg.

‖ LyphoMed®, Melrose Park, IL

Each liter of diet contains (from Multi Vitamin Concentrate): ascorbic acid, 500 mg; vitamin A, 10,000 IU; vitamin D, 1,000 IU; thiamine HCL, 50 mg; riboflavin, 10 mg; pyridoxine HCL, 15 mg; niacinamide, 100 mg; dexpanthenol, 25 mg; vitamin E, 5 IU.

All diets were isovolemic, isocaloric, and isonitrogenous, with a caloric density of 1 Kcal/mL and 5% of total calories as whey protein (Promix R.P.D.®, Navaco Laboratories, Phoenix, AZ). This level of protein, although it appears inadequately low, has been found in previous studies to permit optimal survival in this model.[9]

In the first experiment, three levels of fat were studied in 132 animals: 3.5%, 14%, and 56% of total calories. As the amount of fat in the diets was changed, a corresponding isocaloric change was made in the carbohydrate source (Nutrisource®, Sandoz Nutrition, Minneapolis, MN). Nutrisource® has a caloric density of 3.8 cal/mL, and Microlipid® has a caloric density of 4.5 cal/mL. The MaxEPA® oil was diluted with water to a caloric density of 4.5 cal/mL. Microlipid® also contains soy lecithin (2 g/%) as an emulsifier; therefore soy lecithin (Sherwood Medical) was added at the same concentration to the MaxEPA®.

Also in the first experiment, three different lipid compositions were used (Table 2): 100% Microlipid® (Organon, West Orange, NJ), which is primarily safflower oil; 100% MaxEPA® (R.P. Scherer Corp., Clearwater, FL), which is processed fish oils; and an equal mixture of both oils—the '50:50' group. Microlipid® contains 73.7% omega-6 fatty acids as linoleic acid, and MaxEPA® oil

TABLE 2. *Composition of Experimental Diets*

Experiment One	100% Microlipid	50:50 Mixture	100% MaxEPA®
3.5% of total calories	8 mL Microlipid 0 mL MaxEPA 283 mL Nutrisource	4 mL Microlipid 4 mL MaxEPA 283 mL Nutrisource	0 mL Microlipid 8 mL MaxEPA 283 mL Nutrisource
14% of total calories	30 mL Microlipid 0 mL MaxEPA 251 mL Nutrisource	15 mL Microlipid 15 mL MaxEPA 251 mL Nutrisource	0 mL Microlipid 30 mL MaxEPA 251 mL Nutrisource
56% of total calories	124 mL Microlipid 0 mL MaxEPA 119 mL Nutrisource	62 mL Microlipid 62 mL MaxEPA 119 mL Nutrisource	0 mL Microlipid 124 mL Microlipid 119 mL Nutrisource

Experiment Two		
100% Microlipid*	30 mL Microlipid	0 mL MaxEPA
72/25	22.5 mL Microlipid	7.5 mL MaxEPA
50/50	15 mL Microlipid	15 mL MaxEPA
25/75	7.5 mL Microlipid	22.5 mL MaxEPA
100% MaxEPA	0 mL Microlipid	30 mL MaxEPA

* All diets in experiment 2 had 14% of total calories as fat.

contains 35% omega-3 fatty acids as eicosahexaenoic acid (EPA) and docosahexaenoic acid.[10]

In the second experiment, five different lipid compositions were tested in 58 animals, all at 14% of total calories (Table 2). As before 100% Microlipid®, 100% MaxEPA®, and the 50:50 mixture were used. In addition two other combinations were included, with the two oils mixed in 3:1 ratios, *i.e.*, 75% Microlipid®:25% MaxEPA®, and 25% Microlipid®:75% MaxEPA®.

In the third experiment, three groups fed enteral diets were compared to two groups fed natural guinea pig chow *ad libitum*. Of the two groups allowed to feed on guinea pig chow *ad libitum*, one group was implanted with osmotic pumps filled with sterile saline, thus constituting a sham control group. The three enteral diets were isocaloric and isonitrogenous, with 14% of total calories as fat. These three diets differed only in fat composition, with 100% Microlipid®, 100% MaxEPA®, or a 50:50 combination of both.

In the first and second experiments, animals were fed through gastrostomy for up to 17 days after osmotic pump implantation (up to 14 days of enteral feeding). Survival was observed in these experiments, and in experiment 1 surviving animals were killed 17 days after implantation for studies as described below.

In experiment 3, three groups were fed through gastrostomy for 3 days (up to day 6 after osmotic pump implantation) and two groups were fed chow *ad libitum*. All animals were killed before there was significant attrition in the groups due to death, and studies were performed as described below.

Sacrifice Protocol for Experiment 1

All animals from experiment 1 that survived 14 days after starting the diet (17 days after pump implantation) were killed under general anesthesia by exsanguination from cardiac puncture. Wet weight of the carcass consisting of skeletal and musculofascial structures was measured after the animal was completely skinned, eviscerated, decapitated, and the feet excised. Both gastrocnemius muscles were removed and weighed with the average weight calculated. The liver, spleen, and adrenal glands also were weighed.

Serum albumin concentrations were determined by the bromocresol green method (Gilford Diagnostics, Cleveland, OH). Serum transferrin was measured by laser nephelometry (Immunochemistry Analyzer II®, Beckman Instruments Inc., Fullerton, CA) with appropriate antisera. Serum triglycerides were determined by enzymatic hydrolysis of the triglycerides, followed by colorometric assay of the glycerol (Medical Analysis Systems, Inc., Camarillo, CA). Serum cholesterols were measured by enzymatic hydrolysis followed by spectrophotometric analysis (Medical Analysis Systems, Inc., Camarillo, CA).

Sacrifice Protocol for Experiment 3

Three days after enteral feedings were initiated (6 days after osmotic pump implantation), animals were killed using CO_2 inhalation. When the animals' breathing became agonal, cardiac puncture was done percutaneously and the animals were exsanguinated. Hair was clipped from the ventral thoracoabdominal wall, and the skin painted with povidone-iodine.

A midline incision was made from the sternal notch down to the pubis. Lungs, adrenal glands, liver, spleen, a section of pectoralis muscle, and small intestine were removed. The spleen was weighed and then placed into Hank's buffered salt solution (HBSS). The liver, lungs, muscle, and adrenal glands were weighed, lyophilized, and weighed again. By suspending a standard weight from one end of the gut, creating a uniform degree of tension, a 10-cm segment of proximal jejunum, 10 cm beyond the pyloroduodenal junction, was cut for mucosal sampling. The segment was scraped with a spatula and the wet weight of the mucosa was obtained. A second 10-cm segment beginning 10 cm proximal to the ileocecal valve was similarly prepared.

Immunologic Tests for Experiment 3

Two weeks before gastrostomy, animals underwent sensitization to dinitrofluorobenzene (DNFB). One milliliter of DNFB 0.5% in 5:1 olive oil:acetone was painted on their clipped backs. One day after enteral feedings were begun, animals were challenged with 0.25 mL DNFB 0.5% painted on the right ear after baseline thickness had been measured with calipers. Two days later on the day the animals were killed, ear swelling was measured in inches $\times 10^{-3}$.

Spleens were removed from killed animals to provide lymphocytes and macrophages for analysis. The spleens were minced with forceps and a scalpel and filtered through glass wool to remove debris. The cells were lysed hypotonically and washed twice in HBSS and finally suspended in M199 media containing 100 μg/mL streptomycin, 100 μg/mL penicillin, and 5% heat-inactivated fetal calf serum. The cells were then added to tissue culture plates, incubated for 1 hour to allow adherence of the macrophages, and the nonadherent cells were withdrawn. The macrophages were more than 95% pure as determined with a nonspecific esterase staining kit (Sigma Chemical Co., St. Louis, MO). Viability of the cells was ascertained by trypan blue exclusion.

Nonadherent cells (lymphocytes) were stimulated with 5 μg/mL concanavalin A (Con A), 5 μg/mL phytohemmaglutinin (PHA), or 5 μg/mL pokeweed mitogen (PWM) in tissue culture plates in Roswell Park Memorial Institute 1640 (Gibco, Grand Island, NY) media supplemented with 25 mg/mL nafcillin, 25 mg/mL gentamycin, and 5% nonmitogeneic fetal calf serum in tissue culture plates. After incubation for 24 to 48 hours at 37 C, the cells ($1 \times 10^5/200\ \mu$L) were pulsed for the final 24 hours of culture with [^{3}H]TdR (1.0 μCi/1 $\times 10^5$ cells). The amount of [^{3}H]TdR incorporation was measured in a liquid scintillation counter (Model LS 3155T, Beckman® Instruments, Irvine, CA). Results were expressed as the ratio of mitogen-induced counts per minute (CPM) over control (nonstimulated) CPM.

After withdrawal of the nonadherent cells, the adherent macrophages in the wells were covered over with 1 mL of the M199 medium. To stimulate cultures for prostaglandin assays, *E. coli* lipopolysaccharide (LPS; Sigma Chemical Co.) was added to the culture medium at a final concentration of 20 μg/mL. The macrophages then were cultured for 24 hours in a humidified atmosphere of 5% CO_2/95% air, and the supernatants then were removed and assayed for the various products. All samples for quantitation were frozen at −70 C until assay.

Prostaglandin E_2 enzyme-linked immunoabsorbent assay was performed using PGE_2 tracer and mouse monoclonal anti-rabbit IgG (Caymen Chemicals, Ann Arbor, MI). Cell culture plates with 96 wells each were coated with 2 μg IgG mouse monoclonal antibody in 50 mmol/L (millimolar) KPO_4 buffer per well and incubated at room temperature for 18 hours. The wells were saturated with 200 μg bovine serum albumin (BSA) for at least 18 hours at 4 C before use.

Standards and samples were diluted in EIA buffer (KPO_4 0.1 mol/L (molar), NaN_3 10 mg/%, NaCl 2.34 g/%, Na_4 ethylene diamine tetraacetic acid 37 mg/%, and BSA (100 mg/%), and were added to the plates at 50 μL/well. Tracer and antiserum were added at 50 μL/well. The plates were covered and incubated at 20 C for 18 hours. Enzyme-linked activity was measured by adding 200 μL of color reagent after washing to each well. Readings were taken at 415 nm, and expressed in picograms per milliliter.

Animal Care

In conducting the described research in this report, the investigators adhered to the 'Guide of Laboratory Animal Facilities Care' as set forth by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science—National Research Council and the regulations of the National Institute of Health. The studies were approved by the University of Cincinnati Animal Care Committee.

Statistics

Comparisons of means between groups of continuous variables in experiment 1 was performed with analysis of variance (ANOVA), using type of fat and amount of fat in the diet as the two independent variables. Analysis of sacrifice results from experiment 3 was done with one-way ANOVA. Because PGE_2 values were not normally distributed, they were $\log_e$-transformed before analysis. Correlation between continuous variables was done with simple linear techniques. Analysis of mortality rate was done with the chi square test of independence. Analysis of length of survival was done with nonparametric tests (Kruskal-Wallis ANOVA and Mann-Whitney U test for paired comparisons).

The following *a priori* alternative hypotheses were tested. First that there is a difference between means among the five groups of animals. Second that there is a difference between means of the four septic groups compared to the sham group ('septic' *versus* 'nonseptic'). Third that there is a difference between means of the two groups allowed to feed *ad libitum* compared to the two groups fed enterally *via* gastrostomy ('ad lib' *versus* 'gastrostomy').

An å level of 0.05 was accepted as significant. Calculated probabilities between 0.10 and 0.05 ($0.10 > p > 0.05$) were considered equivocal, particularly because the relatively small numbers of animals in some of the groups preclude significant statistical power. When multiple groups were compared, Fisher's Test of Least Significant Difference was used to evaluate paired comparisons. Statistics were calculated on a Macintosh SE™ computer (Apple Computer, Inc., Cupertino, CA) using Statview 512™ software (BrainPower, Inc., Calabasas, CA).

Results

Length of Survival and Survival Rates—Experiment 1

Length of survival was not significantly affected by the amount of fat in the diet (Kruskal-Wallis ANOVA: $p > 0.1$). However the composition of fat in the diet significantly affected length of survival (Kruskal-Wallis ANOVA: $p < 0.05$). The median day of death in the 100% Microlipid® group was 10 days, in the 50:50 group it was 12.5 days, and in the 100% MaxEPA® group it was 9 days. Analysis by the Mann-Whitney U test showed survival in the 50:50 group to be significantly longer than that in the 100% MaxEPA® group ($p = 0.0110$), but not longer than that in the 100% Microlipid® group ($p = 0.0818$).

Survival rates were analyzed by an overall chi square statistic and were found to differ significantly ($p = 0.0062$). When analyzed by amount of fat in the diet, no differences were seen ($p = 0.1632$). However analysis by type of fat explained the differences seen overall, due to the difference in survival rates between animals fed the 50:50 diets compared to those fed the 100% MaxEPA® diets ($p = 0.0027$). Survival rates from experiment 1 are shown in detail in Table 3.

Sacrifice Results—Experiment 1

There were 30 survivors from all groups in experiment 1. Because in some groups there was only one survivor, statistical comparisons were not done between individual cells. Instead comparisons were made with two-way ANOVA, using the amount and the type of fat in the diets as independent variables. These results are listed in Tables 4 and 5.

Weight change was calculated as the percentage change from the day enteral feedings were started. Although the amount of fat had no significant effect ($p = 0.1688$), the effect of the type of fat was nearly significant ($p = 0.0634$), with apparently less weight loss in the animals fed 100% MaxEPA®.

Weights of carcass, liver, spleen, adrenals, gastrocnemius muscle, and jejunal and ileal mucosal weights were not different among groups, nor was there a difference among groups in serum albumin, transferrin, triglycerides, or cholesterol.

Length of Survival and Survival Rates—Experiment 2

In experiment 2, 58 additional animals were randomized to one of five diets, all with 14% of total calories as fat. The diets differed only in fat composition, using combinations of Microlipid® and MaxEPA®.

Unlike the results of experiment 1, length of survival in experiment 2 was not significantly different among groups (Kruskal-Wallis ANOVA: $0.1 < p < 0.05$). However survival rates again showed significant differences (chi square: $p = 0.0226$; Fig. 1). Survival in the 50:50 group (10 of 12, or 83%) was higher than survival either in the 100% Microlipid® group (2 of 11, or 18%; $p = 0.0068$), or in the 75:25 group (4 of 12, or 33%; $p = 0.0384$). Survival in the 100% MaxEPA® group (4 of 11, or 36%) was lower than that in the 50:50 group to a degree that approached statistical significance ($p = 0.0604$).

Experiment 3 Results

There were 22 animals in the two groups allowed to feed on solid chow *ad libitum*: 13 had osmotic pumps placed that were filled with bacteria and 9 had osmotic pumps placed that were filled with sterile saline. There were 19 animals in the three groups that were fed by gastrostomy: 7 fed diets with 100% Microlipid®, 5 fed diets

TABLE 3. *Experiment 1 Survival Rates*

Lipid Content	100% Microlipid	50:50 Mixture	100% MaxEPA	Amount Subtotal
3.5% of total calories	3/15 20%	2/14 14.3%	1/16 6.3%	6/45 13.3%
14% of total calories	5/15 33.3%	7/15 46.7%	1/14 7.1%	13/44 29.5%
56% of total calories	1/14 7.1%	8/15 53.3%	2/14 14.3%	11/43 25.6%
Type Subtotals	9/44 20.5%	17/44 39%	4/44 9.1%	

TABLE 4. *Experiment 1 Sacrifice Results by Amount of Fat in Diet*

Measurement	3.5% (n = 6)	14% (n = 13)	56% (n = 11)
Weight change (%) p = 0.1688†	−12.3% (4.4)*	−8.4% (2.9)	−18.6% (2.2)
Carcass (g) p = 0.5484	104.727 (7.812)	103.583 (4.953)	90.593 (4.945)
Liver (g) p = 0.6895	13.94 (1.448)	13.399 (0.849)	11.556 (0.875)
Spleen (g) p = 0.5303	0.855 (0.272)	0.642 (0.080)	0.510 (0.044)
Adrenal (g) p = 0.8103	0.337 (0.040)	0.318 (0.024)	0.310 (0.025)
Gastrocnemius (g) p = 0.6416	0.802 (0.058)	0.832 (0.047)	0.753 (0.040)
Jejunal mucosa (g) p = 0.9297	0.459 (0.053)	0.433 (0.021)	0.503 (0.038)
Ileal mucosa (g) p = 0.743	0.276 (0.016)	0.276 (0.023)	0.284 (0.026)
Albumin (g/dL) p = 0.6634	1.6 (0.14)	1.9 (0.23)	2.0 (0.14)
Transferrin (mg/dL) p = 0.1736	35 (6.1)	45 (5.6)	52 (4.5)
Triglycerides (mg/dL) p = 0.3817	40 (8.7)	34 (7.2)	43 (7.2)
Cholesterol (mg/dL) p = 0.3936	77 (37.6)	53 (14.8)	69 (13.8)

* All values given as mean (±SEM).

† Analysis by the variable of amount of fat in two-way ANOVA.

with 50/50 mixture, and 7 fed diets with 100% MaxEPA®. The results are detailed in Table 6.

Weight change from the day that enteral feeding was begun compared to the day of animals were killed was not significantly different among the five groups (p = 0.1624). Wet weights of the spleen, liver, adrenal glands, jejunal and ileal mucosa were not significantly different among the five groups ($p > 0.05$). Wet-to-dry weight ratios of pectoralis muscle, lung, liver, and adrenal glands were not significantly different among the five groups ($p > 0.05$).

Ear swelling response was significantly less in the group fed the enteral diet with 100% Microlipid® compared to all other groups, except for the group fed the 50:50 lipid diet (p = 0.0113). Unstimulated PGE_2 production by splenic macrophages was not significantly different among groups, but LPS-stimulated PGE_2 production was significantly higher in the group fed the enteral diet with 100% Microlipid® compared with all other groups, except for the group fed the 50:50 enteral diet (p = 0.0369). There was a weak but statistically significant negative correlation between the response to DNFB and the $\log_e$-transformed values of LPS-stimulated PGE_2 production (r = −0.382, p = 0.0125).

Lipopolysaccharide-stimulated splenocyte production of PGE_2 correlated with serum levels of oleic acid (r = 0.43, p = 0.007), linoleic acid (r = 0.424, p = 0.0081), and linolenic acid (r = 0.408, p = 0.0111), but not with stearic, arachidonic, or docosahexaenoic acids ($p > 0.05$). Only serum levels of linolenic acid approached significant levels of correlation with response to DNFB (r = 0.296, p = 0.0708).

Phytohemmaglutinin-induced splenic lymphocyte proliferation was equivocally lower (p = 0.0773) in the septic animals allowed to feed *ad libitum*; there were no other statistically significant differences noted among the groups in response to Con A or PWM. Mitogen-induced lymphoproliferation correlated neither with DNFB response nor with splenocyte PGE_2 production.

Effect of Sepsis

The 34 septic animals (implanted with bacteria-filled osmotic pumps) were compared to the nine nonseptic animals (implanted with sterile saline-filled osmotic pumps). Nonseptic animals gained weight during the 3-day observation period before they were killed (+2.3%

TABLE 5. *Experiment 1 Sacrifice Results by Type of Fat in Diet*

Measurement	100% Microlipid (n = 9)	50:50 (n = 17)	100% MaxEPA (n = 4)
Weight change (%) p = 0.0634†	−11.8% (3.9)*	−15.7% (2.2)	−4.6% (4.4)
Carcass (g) p = 0.6897	99.519 (5.478)	101.069 (4.486)	89.401 (11.238)
Liver (g) p = 0.2700	13.751 (1.119)	12.916 (0.693)	10.401 (1.750)
Spleen (g) p = 0.5150	0.737 (0.176)	0.634 (0.069)	0.417 (0.087)
Adrenal (g) p = 0.6212	0.336 (0.034)	0.322 (0.019)	0.269 (0.031)
Gastrocnemius (g) p = 0.9754	0.782 (0.057)	0.820 (0.035)	0.733 (0.074)
Jejunal mucosa (g) p = 0.6638	0.426 (0.038)	0.490 (0.025)	0.436 (0.056)
Ileal mucosa (g) p = 0.5037	0.252 (0.013)	0.293 (0.020)	0.283 (0.048)
Albumin (g/dL) p = 0.4668	1.6 (0.25)	1.9 (0.13)	1.8 (0.43)
Transferrin (mg/dL) p = 0.1800	52 (5.9)	45 (3.9)	34 (11.6)
Triglycerides (mg/dL) p = 0.6157	40 (7.5)	41 (6.3)	25 (8.4)
Cholesterol (mg/dL) p = 0.8730	50 (20.6)	77 (14.9)	38 (11.6)

* All values given as mean (±SEM).

† Analysis by the variable of type of fat in two-way ANOVA.

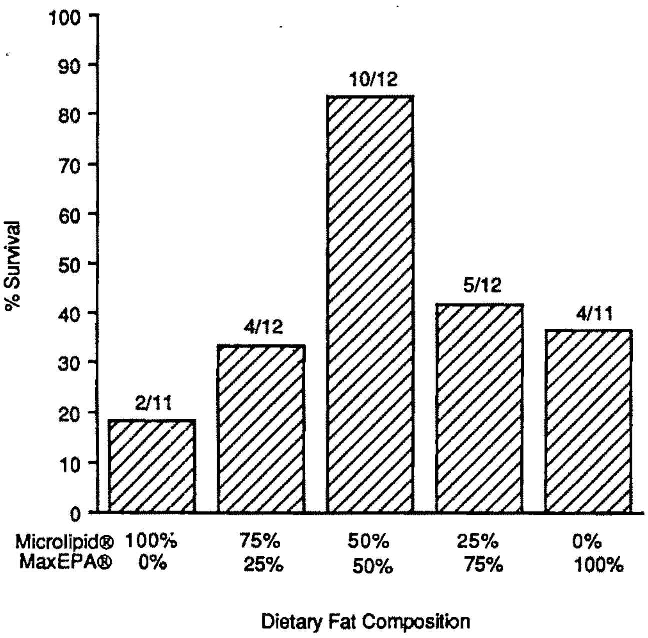

FIG. 1. In experiment 2, all animals were fed diets with 14% of total calories as lipids. The fat composition reflected different combinations of Microlipid® and MaxEPA®. Survival was highest (chi square, p = 0.0226) in the group given equal amounts of Microlipid® and MaxEPA®.

±2.3%), compared to the septic animals, which lost a mean of −4.2% (±1.5%) weight during this period (p = 0.0489).

Although all other tests of immune function were not different between the two groups, lymphocyte proliferation in response to PWM was higher in the nonseptic animals (906 CPM ± 240.6) compared to the septic animals (472 CPM ± 50.1; p = 0.0195). In addition there was an equivocal difference between the mean LPS-stimulated splenocyte PGE_2 production in the septic group (5532.13 pg/mL ± 991.542) compared to the nonseptic group (2294.31 pg/mL ± 685.752; p = 0.0634). There were no other significant differences between the two groups in the measurements performed.

Effect of Feeding Technique

The hypothesis was tested that there was a difference between means of the two groups allowed to feed *ad libitum* compared to the two groups fed enterally through gastrostomy.

Equivocal differences (0.10 > p > 0.05) were noted between the groups fed by gastrostomy and those fed *ad libitum.* Splenic weight was equivocally higher (p = 0.0765) in the groups fed *ad libitum* (0.802 g ± 0.33) compared to those fed by gastrostomy (0.697 g ± 0.05). Wet-to-dry muscle weight ratio was equivocally higher (p = 0.0979) in the groups fed *ad libitum* (6.8 ± 2.8) compared to those fed by gastrostomy (3.6 ± 0.3).

Lipopolysaccharide-stimulated splenocyte production of PGE_2 was equivocally higher (p = 0.076) in the group fed by gastrostomy (5688.45 pg/mL ± 981.09) compared to those fed *ad libitum* (4058.34 pg/mL ± 1298.305). Although other tests of lymphocyte proliferation did not differ *vis á vis* route of feeding, lymphocyte proliferation in response to PHA was equivocally higher (p = 0.0708) in the gastrostomy-fed animals (1363 CPM ± 249.0) compared to the animals fed *ad libitum* (839 CPM ± 169.2). There were no other significant differences between the two groups in the measurements performed.

Discussion

These experiments were designed to test the dual hypothesis that both amount and type of fat in enteral diets can influence outcome in an animal model of peritonitis. The results indicate that the amount of fat in the diet has no impact on survival in this model, nor does it appear to affect carcass or organ weights, or serum levels of albumin, transferrin, triglycerides, or cholesterol in surviving animals.

However comparison of survival among the groups fed different combinations of vegetable and fish oils showed significant differences. In both experiments 1 and 2, survival rates were highest in the group fed equal amounts of Microlipid® and MaxEPA® oils. In addition the length of survival in experiment 1 was significantly longer in the group fed the 50:50 mixture, although this was not seen in experiment 2. Examination of carcass and organ weights of the survivors of experiment 1 showed no differences among groups. Similarly serum albumin, transferrin, triglycerides, and cholesterol were not different.

Data gathered from the animals killed after 3 days on the enteral diets show that PGE_2 production by splenic macrophages was increased in animals fed diets with safflower oil-based enteral diets. Also cell-mediated immunity, as measured by the response to DNFB, was decreased in these animals. There was, in fact, a weak but statistically significant negative correlation between splenocyte PGE_2 production and response to DNFB. However these results do not serve to explain the observation that diets with equal amounts of fish and safflower oil (with an omega-6:omega-3 ratio of approximately 2:1) were associated with the best outcome in this guinea pig model of sepsis.

Previous studies also have shown that dietary fat manipulation can influence outcome from infection or injury. Guinea pigs recovering from 30% total body surface area (TBSA) flame burns were enterally fed isocaloric and isonitrogenous diets differing only in lipid type. When compared to those fed safflower oil (74% linoleic acid) or linoleic acid alone, the animals fed fish oil had less weight loss, better skeletal muscle mass, lower resting metabolic expenditure, better cell-mediated immune responses, bet-

TABLE 6. *Experiment Three Sacrifice Results*

Measurement	Microlipid 100%	50:50	MaxEPA 100%	*Ad lib* Infected	*Ad lib* Sham
% Δ weight p = 0.1624	−3.2% ± 4.2	+1.1% ± 4.5	−6.6% ± 2.8	−5.1% ± 2.1	+2.3% ± 2.3
Spleen (g) p = 0.1513	0.593 ± 0.051	0.773 ± 0.087	0.757 ± 0.101	0.805 ± 0.045	0.798 ± 0.052
Liver (g) p = 0.4379	12.183 ± 1.503	14.953 ± 2.393	15.370 ± 1.182	14.442 ± 0.912	15.888 ± 1.541
Adrenals (g) p = 0.7727	0.333 ± 0.036	0.362 ± 0.048	0.357 ± 0.039	0.329 ± 0.028	0.306 ± 0.015
Jejunal mucosa p = 0.7313	0.274 ± 0.025	0.312 ± 0.052	0.328 ± 0.048	0.257 ± 0.031	0.304 ± 0.055
Ileal mucosa p = 0.5918	0.226 ± 0.025	0.280 ± 0.066	0.233 ± 0.045	0.202 ± 0.027	0.193 ± 0.022
Muscle wet/dry p = 0.3226	3.4 ± 0.44	4.5 ± 0.53	3.0 ± 0.81	8.5 ± 4.73	4.1 ± 0.52
Lung wet/dry p = 0.3017	5.0 ± 0.47	5.4 ± 0.15	4.9 ± 0.36	5.5 ± 0.32	3.8 ± 1.23
Liver wet/dry p = 0.7034	3.7 ± 0.14	3.8 ± 0.07	3.6 ± 0.22	3.4 ± 0.25	3.4 ± 0.04
Adrenal wet/dry p = 0.1662	3.0 ± 0.21	3.2 ± 0.25	3.2 ± 0.51	2.4 ± 0.22	4.2 ± 1.6
DNFB (in × 10^{-3}) p = 0.0113	7.2 ± 2.09	13.4 ± 1.25	20.4 ± 2.52	16.6 ± 2.20	16.8 ± 2.06
PGE_2/M199* p = 0.5417	141.11 ± 38.846	98.70 ± 13.584	383.97 ± 228.767	196.70 ± 56.319	136.86 ± 18.552
PGE_2/LPS* p = 0.0369	8466.75 ± 1810.017	5777.40 ± 2141.412	3478.14 ± 1033.550	5279.60 ± 2144.498	2294.31 ± 685.752
PHA (CPM)† p = 0.0773	1558 ± 459.2	1767 ± 829.5	1079 ± 285.5	516 ± 87.0	1242 ± 320.1
PWM (CPM)† p = 0.111	544 ± 146.4	679 ± 221.3	447 ± 54.7	400 ± 74.7	906 ± 240.6
Con A (CPM)† p = 0.1588	1666 ± 376.6	2241 ± 1200.9	1819 ± 228.3	1183 ± 265.6	2325 ± 479.2
Control (CPM)†	460 ± 260.8	447 ± 113.7	307 ± 53.4	203 ± 32.4	370 ± 153.9

All results listed are group means ± SEM.

* PGE_2 values are in picograms/mL, and were $\log_e$ transformed before analysis.

† Results of lymphocyte proliferation are expressed here as mean (±SEM) counts per minute (CPM). However statistical analysis was done on the $\log_e$ transformed ratio of stimulated/unstimulated CPM (*i.e.*, In [PHA/control], in [PWM/control], and in [Con A/control]).

ter opsonic indices, higher splenic weight, lower adrenal weight, higher serum transferrin, and lower serum C_3 levels.[11]

In other studies omega-6 fatty acids have been shown to alter the response to endotoxin. Cook and associates[12] found that rats on essential fatty acid-deficient diets had only a 24% mortality rate after endotoxin challenge, but mortality rate increased to 100% when arachadonic acid was given 2 days before challenge.

In addition omega-3 fatty acids may ameliorate the response to endotoxin. Mascioli and associates[13] fed guinea pigs for 6 weeks diets with 36% of calories as either safflower oil or menhaden oil, which is high in omega-3 fatty acids. Menhaden oil-fed animals had significantly higher survival rates at 20 hours after intraperitoneal endotoxin challenge than animals fed safflower oil.

Contradictory results have come from experiments using burned mice that were subsequently infected. Mice were fed for 2 to 3 weeks on diets with different amounts and types of fats, including fish oil and safflower oil. They then were subjected to a 20% TBSA flame burn and challenged with *Pseudomonas aeruginosa* subeschar. Survival in the group fed a diet high in safflower oil (40% of total calories) was significantly better than that in the group fed a diet high in MaxEPA®.[14]

Thus there is evidence in the literature to support the hypothesis that omega-3 fatty acids can improve outcome from burn or endotoxin challenge in guinea pigs. However, in a more complex murine model of injury and infection, there appears to be a distinct advantage to omega-6 fatty acids. Our results from this study would suggest that both families of fatty acids are important in recovery from peritonitis in guinea pigs.

We conclude that although the amount of fat fed enterally to guinea pigs during bacterial peritonitis does not affect outcome, the composition of fat has a very strong influence. It is not clear, either from previous studies in this laboratory and others, or from examinations of the surviving animals in this study, what mechanisms are responsible for this effect. Further studies are needed to determine how changes in eicosanoid production and immune function affect the improvement in survival that is noted with diets composed with both fish and vegetable oils.

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Anti-tumor Effect of L-methionine–Deprived Total Parenteral Nutrition with 5-Fluorouracil Administration on Yoshida Sarcoma-bearing Rats

NARIHIDE GOSEKI, M.D.,* MITSUO ENDO, M.D.,* TOKIO ONODERA, M.D.,† and GORO KOSAKI, M.D.†

L-methionine–deprived total parenteral nutrition (methionine-deprived TPN), infusing amino acid solution devoid of L-methionine and L-cysteine by the method of TPN as an only protein source, showed enhancement of the effect of several anti-cancer agents. In this study the combined effect of the methionine-deprived TPN with administration of 5-fluorouracil (5-FU) was examined in Yoshida Sarcoma (YS)-bearing rats, from aspects of effects on the tumor metastasis and the host animal's life span, in the following four groups treated with: methionine-deprived TPN with administration of 5-FU, methionine-deprived TPN without administration of 5-FU, L-methionine–contained TPN plus 5-FU, and L-methionine–contained TPN without 5-FU. In the first experiment, TPN was continued for 8 days in the four groups, and the anti-cancer effect of methionine-deprived TPN and administration of 5-FU based on both the growth of the primary tumor at the implanted site and the tumor metastasis was studied from the view point of pathologic findings of animals killed immediately after these treatments. In experiment 2 the survival period was examined after these treatments for 10 days with subsequent oral feeding until death. The results were as follows: proliferation of YS, transplanted subcutaneously, was markedly suppressed; particularly hematogenous metastasis, characteristic in YS, was prominently blunted, then obtained an apparent longer survival period in rats treated with the methionine-deprived TPN with administration of 5-FU.

From the First Department of Surgery, Tokyo Medical and Dental University, School of Medicine, and the Department of Surgery,† Tokyo Metropolitan Komagome Hospital, Tokyo, Japan*

L-METHIONINE, A sulfur-containing amino acid, is essential for methylation in the synthesis of RNA, DNA, protein, and other biochemical substances. In tissue culture studies, it was demonstrated that the various malignant tumor cells were unable to proliferate in a medium deprived of L-methionine.[1–5] Kreis et al.[4] reported that tumor growth was suppressed in rats implanted with Walker carcinosarcoma with transient decrease of methionine level in the body by enzyme. In our previous experiments, we were able to decrease safely the level of methionine in the body using an amino acid mixture lacking in L-methionine and L-cysteine in total parenteral nutrition (methionine-deprived TPN),[6] and this parenteral therapy has been studied as an adjunct to cancer chemotherapy.[7–11] In an experiment with dogs that were subjected to this therapy for 3 weeks, methionine starvation was admitted well enough, without particular adverse side effects, except a decline in the nutritional state to some degree, by this treatment.[6] Furthermore, in the experiments with rat-bearing AH-109A tumor (ascites type), it was demonstrated that methionine-deprived TPN showed an apparent anti-tumor effect.[11] On the other hand, in the experiment of Sato, in lung carcinoma-(solid type) bearing rats, the anti-cancer effect was not so apparent, although L-methionine levels both in tumor and hepatic tissue were markedly reduced.[12] However methionine-deprived TPN with several anti-cancer agents enhanced the anti-tumor effect of almost all of these agents and this enhancement was followed by the suppression of proliferation of Sato lung carcinoma (SLC), without serious adverse side effects in the host animals.[13,14] Therefore it can be proposed that this unbalanced amino-acid solution may be substantially related to the metabolism of various cancer agents, resulting in a synergistic effect when used with suitable anti-cancer agents. Thus this therapy involves as a mechanism, a remarkable delay in turnover of the tumor cell[15] and a marked decline of thiol levels in hepatic and tumorous tissue.[13]

Recently Breillout et al.[16] reported that a methionine-deprived diet suppressed metastasis of a transplantable tumor in mice. Methionine is the precursor of S-adenosyl methionine, which is major methyl group donor in transmethylation; methylation of cell membrane phospholipids

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Address reprint requests to Narihide Goseki, M.D., The First Department of Surgery, Tokyo Medical and Dental University, School of Medicine, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113, Japan.

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and DNA is related to metastatic ability of tumor cells. Accordingly we studied the effect of a combination therapy with methionine-deprived TPN and 5-fluorouracil (5-FU) on the metastasis of Yoshida sarcoma (YS), which metastasizes through the blood stream when administered subcutaneously. In conclusion hematogenous metastasis of the tumor was suppressed and, as a result, a marked elongation of life span was observed in the host rats.

Materials and Methods

Experiment 1

The growth inhibitory effect of combination therapy of methionine-deprived TPN and administration of 5-FU on YS was examined.

Six-week-old male Donryu rats, weighing 163 ± 7.0 g, obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan) were used for the experiment. Rats were transplanted with 1×10^6 cells of YS to dorsal adipose tissue (day 0), which made the solid-type tumor. The tumor cells (obtained from Sasaki Institute, Tokyo, Japan) were maintained as ascites tumor in Donryu rats by weekly intraperitoneal implantation. On the seventh day of transplantation, tumor cells were sampled to confirm the number of viable cells using the trypan blue exclusion test. Rats were cannulated to the vena cava immediately after tumor transplantation according to the method of Steiger et al.,[17] and were maintained on TPN with one of the four regimens combined with or without 5-FU. The amino acid solutions used in TPN were AO-90, which does not contain L-methionine and L-cysteine (Table 1) and commercial Pan-Amin S®, from which the AO-90 was prepared by removing L-methionine. The rats were not allowed to take any foods orally during the TPN period. The animal groups were as follows:

1. The AO-90 plus 5-FU group (n = 6) received AO-90 for 8 days. Total parenteral nutrition regimens were shown in Table 2. 5-Fluorouracil was mixed with the TPN solution and infused for 6 days (from day 2 to 7) at a dose of 15 mg/kg/day (90 mg/kg total).
2. The AO-90 group (n = 5) received TPN with AO-90 for 8 days without 5-FU administration.
3. The Pan-Amin S plus 5-FU group (n = 6) received TPN for 8 days with Pan-Amin S®, which contains L-methionine. 5-fluorouracil was infused in the same dose and manner as in the AO-90 plus 5-FU group.
4. The Pan-Amin S group (n = 5) received TPN for 8 days with Pan-Amin S® without 5-FU.

TABLE 1. *Compositions of Amino Acid Solutions*

Amino Acid	AO-90 (g/dL)	Pan-Amin S® (g/dL)
L-Arginine	0.66	0.66
L-Histidine	0.30	0.30
L-Isoleucine	0.55	0.55
L-Leucine	1.23	1.23
L-Lysine	1.49	1.49
L-Methionine	—	0.71
L-Phenylalanine	0.87	0.87
L-Threonine	0.54	0.54
L-Tryptophan	0.18	0.18
L-Valine	0.61	0.61
Glycine	1.00	1.00
Total amino acids (g/dL)	7.43	8.14
Total N (g/L)	11.9	12.6

TABLE 2. *Composition of TPN Solution (amount daily/kg)*

TPN Solution	Pan-Amin S	AO-90
50% Glucose (mL)	105	105
Pan-Amin S (mL)	123	—
AO-90 (mL)	—	123
Electrolyte sol. (mL)	25.6	25.6
Sohvita* (mL)	0.1	0.1
Total volume (mL)	254	254
Glucose (g)	52.5	52.5
Amino acids (g)	10	9.14
Na (mEq)	19.0	19.0
K (mEq)	2.6	2.6
Ca (mEq)	1.1	1.1
Cl (mEq)	19.0	19.0
Mg (mEq)	1.1	1.1
HPO_4 (mEq)	2.6	2.6
Total calories (Kcal)	250	247
Total N (g)	1.55	1.46
Nonprotein calories/N	135	144

* Amounts per 254 mL of infusate: thiamine HCl, 0.125 mg; riboflavin sodium phosphate, 0.125 mg; pyridoxine HCl, 0.075 mg; cyancobalamin, 0.75 μg; nicotinamide, 0.5 mg; folic acid, 0.025 mg; biotin, 5 μg; ascorbic acid, 2.5 mg; panthenol, 0.3 mg; retinol palmitate, 62.5 IU; cholecalciferol, 5 IU; tocopherol acetate, 0.375 mg; menatetrenone, 0.05 mg.

TPN, total parenteral nutrition.

During TPN the rats were housed individually in metabolic cages. A microinfusion pump was used for constant administration of TPN solution. At the end of the experiment (day 8), rats were killed and the tumor-implanted area was extracted to measure the tumor and carcass weight. Then major organs, including the liver and lungs, were histopathologically examined for the presence of tumor cells.

Experiment 2

Life span of YS-bearing rats administered with the methionine-deprived TPN combined with the anti-cancer agent 5-FU was studied.

To examine the synergistic effect of the methionine-deprived TPN toward 5-FU, 8-week-old male Donryu rats (Shizuoka Laboratory Animal Center) weighing 257 ± 13 g were transplanted with 1×10^4 cells of YS into the dorsal adipose tissue as in the experiment 1, and then cannulated vena cava for TPN procedure (day 0). Experimental design is shown in Figure 2. There was slight variation in both the dosage of 5-FU and a section of the administration method, but otherwise the procedure was the same as that for experiment 1. The groups were AO-90 plus 5-FU (n = 6), AO-90 (n = 5), Pan-Amin S plus 5-FU (n = 6), and Pan-Amin S (n = 7). The AO-90 plus 5-FU and the Pan-Amin S® plus 5-FU received 10 mg/kg/day of 5-FU (80 mg/kg total) mixed in TPN solution for 8 days, and also given 10 mg/kg/day intraperitoneally, twice, on days 10 and 11. These tumor-bearing rats were, as in experiment 1, housed individually in metabolic cages and maintained on TPN for 10 days. After TPN the animals were fed a laboratory chow (Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum* to survey their life span. Then all of the rats were studied, pathohistologically, at necropsy to determine tumor metastasis and cause of death.

We started with six rats in each group of experiment 1 and seven rats in experiment 2. Because those animals that had a problem of solution leakage from the catheter were excluded from the study, the number of rats in each group decreased.

To examine the tumor extension in rats when they were killed (experiment 1) or at death (experiment 2), all animals were surveyed pathologically and the following criteria were used.

(−): No evidence of metastasis with histologic examination.

(±): With histologic examination only a small amount of tumor cells were observed.

(+): With histologic examination small foci of tumorous formation of cancer cells were demonstrated in the examined organs.

(++): With histologic examination, the apparent tumor metastasis-forming masses were demonstrated, but no more than one half of the organ tissue.

(+++): With pathologic examination marked metastasis of the examined organs was demonstrated and was thought to be due to the metastasized organ's failure.

Results

Experiment 1

Tumor weight (Fig. 1). Tumor weight, carcass weight, and tumor weight:carcass weight ratio on day 8 (at the end of the experiment) were 0.69 ± 0.14 g, 153 ± 7.0 g, and 0.5% ± 0.1%, respectively, for the AO-90 plus 5-FU group, 1.62 ± 0.51 g, 153 ± 8.0 g, and 0.7% ± 0.37% for the AO-90 group, 1.64 ± 0.40 g, 174 ± 13 g, and 0.93% ± 0.21% for the Pan-Amin S plus 5-FU group, and 3.13 ± 0.50 g, 174 ± 10 g, and 1.79% ± 0.24% for the Pan-Amin S group. Tumor weight and tumor:carcass weight ratio were significantly lower in the AO-90 plus 5-FU group than other groups at $p < 0.01$ by Student's t test.

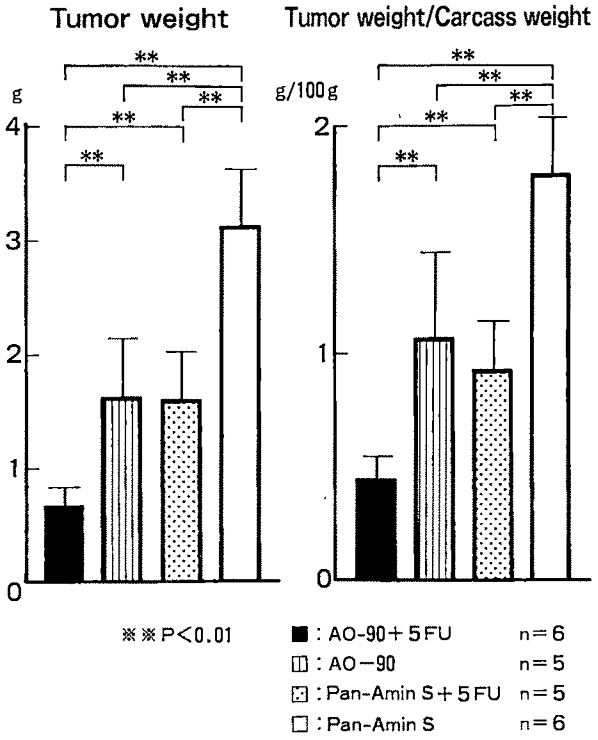

FIG. 1. Illustration of the results of experiment 1 (tumor weight and tumor weight/carcass weight shown in each group).

Pathohistologic findings (extension of tumorous lesions). Pathologic findings at the end of the experiment are shown in Table 3 with the above-mentioned criteria.

In the AO-90 plus 5-FU group, only one rat showed hematogenous liver metastasis, and in the AO-90 group, only one rat had metastasis in the liver, the lungs, and the kidneys. Other rats in these two groups showed no hematogenous metastasis with histopathologic examination. On the other hand, in the Pan-Amin S plus 5-FU and the Pan-Amin S groups, all rats developed severe hematogenous metastasis in several organs.

Experiment 2

Survival days (Fig. 2). Mean survival days were 45.2 ± 17.6 for the AO-90 plus 5-FU group (two rats were killed on day 60), 30.4 ± 18.0 days for the AO-90 group (one rat was killed on day 60), 21.3 ± 8.4 days for the Pan-amin S plus 5-FU group, and 15.9 ± 3.8 days for the Pan-Amin S group. The AO-90 plus 5-FU group had a significantly longer survival period than the Pan-Amin S plus 5-FU ($p < 0.05$) and the Pan-Amin S ($p < 0.01$) (Wilcoxon's rank sum test) groups.

Histologic findings at autopsy (Table 4). In Table 4 the tumor extension of each rat at death was summarized following the above-mentioned pathohistologic findings with the five criteria, which were also used in experiment 1, with other findings such as tumor weight, survival pe-

TABLE 3. *Pathologic Findings of Tumor Metastasis in Each Rat of Four Groups at Autopsy*

Animal No.	Group	Metastasis				
		Liver	Kidney	Lung	Heart	Spleen
1	AO-90 + 5-FU	−	−	−	−	−
2		++	−	−	−	−
3		−	−	−	−	−
4		−	−	−	−	−
5		−	−	−	−	−
6		−	−	−	−	−
7	AO-90	−	−	−	−	−
8		±	−	−	−	−
10		−	−	−	−	−
11		±	−	±	−	−
12		+	+	+	−	−
13	Pan-Amin S + 5-FU	++	+	+	−	±
15		++	+	−	−	−
16		++	−	+	−	−
17		+	−	++	+	−
18		+	+	+	−	−
19	Pan-Amin S	+++	−	++	−	++
20		+++	−	+++	+	++
21		++	−	++	−	±
22		++	±	++	−	−
23.		±	−	++	−	−
24		±	−	++	−	+

−, ±, +, ++, +++: cf. text.

riod, and so on. With the examination of the tumorous area in death cases, two rats (33.3%) in the AO-90 plus 5-FU group the implanted tumor was decreased in severe necrosis and completely diminished from the implanted site, while only three rats (50%) had slight hematogenous metastasis. The cause of death in this group was organ failure: death induced by respiratory and hepatic failure attributable to hematogenous metastasis in two rats (33.3%); the other two rats (33.3%) probably died of voluminous bleeding body fluid loss or infection from the necrotic lesion of the remnant tumor of the implanted site.

The cause of death seen in the AO-90 group was organ failure resulting from tumor metastasis in three rats (60%) and bleeding from primary tumor lesion in one rat (20%), while the tumor in one rat (20%) was eradicated.

In the Pan-Amin S and the Pan-Amin S plus 5-FU groups, one rat (8.3%) died of body fluid loss, bleeding from primary tumor, and accompanying infection. The other 11 rats (91.7%) were thought to have died of organ failure due to severe hepatic and pulmonary hematogenous metastasis of the tumor.

Discussion

Increase or deprivation of certain amino acids, *i.e.*, amino acid imbalance, has been reported to induce the inhibitory effect to tumorous proliferation. In particular methionine is documented to be essential for growth of tumor cells. Many tumor cells, including L5178 murine leukemic cells, are difficult to proliferate in a methionine-free medium in comparison with the normal fibroblast.[1-5] In addition, when the L-methionine level was forced to decrease transiently by the enzyme such as L-methioninase in rats transplanted with Walker carcinosarcoma, the proliferation of tumor cells was blunted.[4]

In our recent studies, a special unbalanced amino-acid solution (AO-90), devoid of all the sulfur-containing amino acids, was prepared by eliminating L-methionine and L-cysteine. This solution was infused safely by TPN method to develop methionine starvation in the body, together with minimizing deterioration of nutritional condition.[6] This TPN method was investigated with regard to the influence on body metabolism and anti-tumor effect.[7-13]

Because 50% of nontumor-bearing rats died after the 3-week AO-90 regimen, we administered the TPN solution for 8 days in the experiments. In our experiments

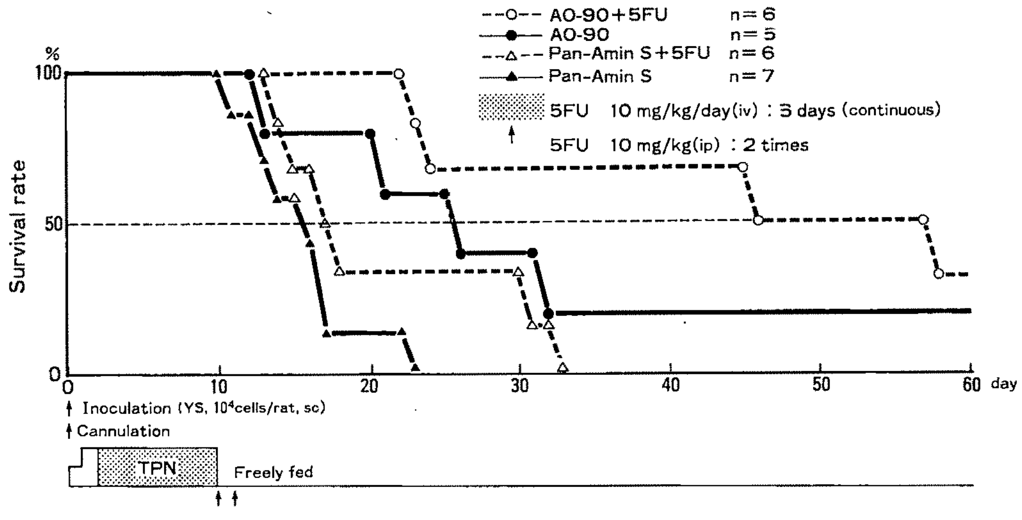

FIG. 2. Illustration of the protocol of experiment 2 and its results (survival rates and days of the rats are shown in each group).

TABLE 4. *Pathologic Findings of Tumor Metastasis at Autopsy, Tumor Weight, Survival Days, Cause of Death and Other Findings in Each Animal of Four Groups*

Group	Animal No.	Metastasis: Liver	Kidney	Lung	Heart	Spleen	Tumor Weight (g)	Survival Days	Other Findings	Cause of Death
AO-90 + 5-FU	1	−	−	−	−	−	None*	<60		None
	2	+++	+++	++	−	−	18.6†	23	Peritoneal dissemination	Meta§
	3	−	−	−	−	−	82.4†	46		Tumor‖
	4	−	−	+	−	−	79	58		Tumor
	6	±	−	−	−	−	None*	⩾60		None
	7	+	+++	+++	+++	−	16.3	24	Pleuritis	Meta
AO-90	8	−	−	−	−	−	None†	>60		None
	10	+++	+++	++	−	−	2.0	13		Meta
	11	−	−	−	−	−	24.1	32	Peritoneal dissemination	Tumor
	12	+++	+++	+++	−	−	25	26	Pleuritis	Meta
	14	++	++	+++	−	−	17.6	21	Pleuritis	Meta
Pan-Amin S + 5-FU	16	−	++	+++	−	+++	8.2‡	33	Peritoneal dissemination	Meta
	17	+++	+++	+++	++	−	8.1	15		Meta
	18	++	+++	+++	−	−	11.9	17		Meta
	19	++	+++	+++	++	−	10.9	18		Meta
	20	——	——	——	——	——	48.6	31	Peritoneal dissemination	Meta
	21	+++	+++	+++	+	−	12.8	14		Meta
Pan-Amin S	22	+	−	−	−	−	24.1	23		Tumor
	23	++	+++	+++	+++	−	7.2	16		Meta
	24	++	++	+++	++	−	5.4	14		Meta
	25	−	−	+++	−	−	13.7	17		Meta
	26	−	−	+++	−	−	13.9	17		Meta
	27	+++	++	+++	++	−	3.1	11		Meta
	28	+++	+++	+++	−	−	7.1	13		Meta

−, ±, +, ++, +++: cf. text.

* Tumor in the transplanted area was fall in severe necrosis and diminished from the host animal at autopsy.

† Showed complete regression of the tumor.

‡ Most part of the tumor was fall in necrosis macroscopically.

§ Meta, pathologically diagnosed to be dead of the metastasized lesion.

‖ Tumor, pathologically diagnosed to be dead of bleeding, loss of body fluid and/or infection from the transplanted area's tumor.

the growth of YS did not depend on nutritional regimen to the host animals, whether it was TPN or chow feeding, when calorie intake was limited to not more than 250 kcal/kg. Accordingly we did not include a group of orally fed tumor-bearing rats as another control group. The inoculum size of the tumor cell was different in experiment 1 from that of experiment 2. In general 10^6 YS cells are inoculated in this kind of experiment. In experiment 1 we inoculated this number of tumor cells. However this amount is too large to demonstrate a life-expanding effect of the regimen because the animals inoculated with this amount of tumor cells die quickly. For this reason we inoculated 10^4 cells in experiment 2.

As a result proliferation of YS was markedly suppressed by the methionine-deprived TPN. Hematogenous metastasis, which is characteristic to the tumor, was prominently blunted as well. These effects of the regimen apparently resulted in a longer survival period in rats treated with the methionine-deprived TPN with administration of 5-FU.

As we previously reported,[13] nimustine hydrochloride to which SLC is sensitive, exerted the increased anti-tumor effect in rats because of a marked decline of the thiol level in tumorous tissue as well as in the liver.

And in the rats bearing AH-109A tumor cells, the metabolism of nucleic acid in the tumor was altered, as demonstrated by enlargement of nuclear size with accumulation of RNA granular (probably by inhibition of r-RNA processing).[11] Furthermore, in the same rat model, the cell cycle of the tumor has been found to be delayed markedly, as seen from the analysis of fraction-labeled mitosis curve.[15]

However it appears that these observations cannot satisfactorily explain the synergistic increase of anti-tumor effect of cancer agents by concurrent use of methionine-deprived TPN. In fact the effect of methionine-deprived TPN itself was relatively prominent in the AH-109A tumor[11] but unexpectedly was not effective on solid SLC tumors.[12] For example life span was lengthened in the AH-109A–bearing rats when actinomycin D was combined,[8] whereas longer survival time was not achieved in the SLC-bearing rats, although anti-tumor effects were clearly recognized.

In the present study with YS cells that easily induce metastatic organ failure, followed by death with extensive hematogenous metastasis, methionine-deprived TPN was demonstrated to reduce markedly the degree of metastasis, resulting in lengthened survival with combined use with 5-FU.

Breillout et al.[16] reported that a methionine-deprived diet inhibited metastatic spread of Lewis lung carcinoma and rhabdomyosarcoma tumor cells grafted into animals. They could hypothesize that the metastatic process is easily disturbed by a methionine-deprived diet, affecting the cell membrane phospholipid and DNA methylation. In the same way, AO-90 might inhibit metastatic processes. And the metastases appear to be more receptive of this therapy than do primary tumors.[18] But the chemosensitizing effect to the 5-FU by AO-90 is not fully explainable.

To elucidate the mechanism, it is necessary to make a further fundamental examination. As to possible unfavorable influences by the therapy which concern much in the study, only slight decline of serum protein level was noted in dogs rendered to a 3 week TPN as well as in the clinical pilot trial and phase I study.[8,9,14]

Now it is very necessary to examine further the effect of this unbalanced amino acid solution in combination with various anti-cancer agents and also to elucidate the involving mechanism from the various points. We add herein that clinically effective cases have been seen in advanced gastrointestinal cancer patients.

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BOOK REVIEWS

Mysterious Heparin: The Key to Open Heart Surgery. Wilfred G. Bigelow. 205 pp. Toronto: McGraw-Hill Ryerson, 1990. $32.95.

THIS IS A VERY engaging and informative monograph written by a pioneer and major contributor in the development of cardiac surgery. Wilfred G. Bigelow's role in the field of hypothermia is recognized worldwide and continues as a very important aspect of open heart surgery. Hypothermia has become an extremely significant part of the armamentarium both in providing ideal circumstances for appropriate technical correction of cardiac defects and also in the reduction of the number of operative deaths.

Quite early it was recognized that extracorporeal circulation was achievable by use of heparin for systemic anticoagulation. In this praiseworthy monograph, the author describes the events that provided the stimulus for preparing this text that occurred during an international surgical symposium in 1986. Bigelow was asked to join other pioneers in this session devoted to a broad review of open heart surgery. Many years ago he simply stated, '*Heparin* was the thread that tied all the cardiovascular developments of 50 years into a unified whole.'

This monograph is designed to attract a broadly based audience and therefore is written in a style easily read by laymen and scientists alike. It begins with the experiences of a medical student, Jay McLean, while working in the laboratory of William Henry Howell, the Professor of Physiology at Johns Hopkins. Howell gave McLean the project of identifying an agent that would increase the clotting of the blood and be effective in the control of hemorrhage. He pursued this advice and identified an active extract of the brain, principally cephalin, which stimulated the clotting of blood. He kept the extracts of the brain as well as those of heart and liver, and found that with the passage of time they lost their potency. Furthermore, being preserved, liver extract actually prevented clotting. This was the initial discovery of heparin and represented a major scientific landmark.

The author also describes the development of insulin, through the work of Banting and Best in 1921 and 1922. Best was a medical student at the time and was later selected for the Chair in Physiology at the University of Toronto, succeeding the famed Professor J. J. R. MacLeod. He then describes the initial use of heparin clinically by Gordon Murray, a surgeon who made many fundamental contributions in the experimental laboratory. At about this time, Clarence Crafoord, a Swedish surgeon, became interested in emergency pulmonary embolectomy for removal of emboli from the pulmonary arteries. Crafoord recognized that there was no simple therapy once a large embolus had reached the lung, and his knowledge of the field made him aware of the need for an anticoagulant to prevent blood clot formation.

The story of heparin is made vibrant through presentation of all aspects of its development and use. He describes the work performed at the Connaught Laboratories, a distinguished Canadian pharmaceutical firm, and a part of the School of Hygiene of the University of Toronto.

Wilfred G. Bigelow, one of Canada's most outstanding leaders, is the recipient of 17 major awards, including the Order of Canada. Known worldwide for his research contributions with the development of hypothermia, he also had a key role in the design of pacemakers. Since retirement he has devoted himself to scholarly writing and also working to protect the environment in Canada.

In the final chapter, the author devotes attention to the reason he has called heparin 'a mystery.' One facet of this is the fact that the agent has not yet been synthesized because there is not an assigned formula. Rather heparin is composed of more than 100 related substances clinging together as a unit. The exact chemical description of heparin remains a bold challenge to scientists and much further investigation will be necessary to identify the pure fraction of heparin that prevents blood clotting. Progress is being made in the development of low–molecular-weight heparin, which has been achieved by separation of a fraction of a mixture of molecules that constitute heparin.

From the beginning of this intriguing monograph, the reader's interest is held tightly to the text. The author is a very skillful writer who presents one feature of heparin after another in a very enticing manner. Just as the text begins with the reader's committed attention, so it ends with challenging comments and creates continuing interest in this important field by stating, 'So, after all these years of research, medical scientists may be embarking on further new adventures in the study of heparin's components. This is the way of research and progress, which confirms the old adage: each end is a new beginning.' Quite predictably, this text will be widely read by laymen and scientists alike and both will profit greatly from its message.

DAVID C. SABISTON, JR., M.D.
Durham, North Carolina

Vascular Surgery: A Comprehensive Review, 3rd Edition. Wesley S. Moore. 792 pp. Philadelphia: WB Saunders, 1991. $125.00.

IN 1983, Dr. Wesley Moore and colleagues at the University of California in Los Angeles initiated a comprehensive review course in vascular surgery. This was the first year the American Board of Surgery administered an examination for Certification of Special Competence in General Vascular Surgery. Consequently Moore et al. wanted to provide a course focused on updating practicing surgeons and on preparing them to pass the examination.

They must have succeeded. The course remains immensely popular to this day, and the accompanying textbook has reached its third edition in only 7 years. Initial strengths of the course and the text included the comprehensive content, excellent contributors, and study questions. As the authors perpetuate their efforts, they must not lose sight of these early ingredients to their success.

The current text is again comprehensive. All major vascular topics are covered with timely rewriting of old chapters and the introduction of new material. Important additions include chapters on vascular wall biology and endovascular surgery. The text is also strengthened by contributions from several vascular radiologists and cardiovascular internists.

The third edition continues to rely on the input of well-known experts in the field of vascular disease and surgery. They obviously provide a certain diversified credibility to the viewpoints on each topic. Most of them have carefully updated their original contributions to the second edition.

If the third edition falters in any area, it does not consistently provide study questions for each chapter. These questions were a unique and useful feature of previous editions. Some of the current questions seem hurriedly constructed and perhaps not validated by standard examination techniques. If space limitations constrain the inclusion of study questions for this new edition, extra space could easily be freed up by distilling the reference lists, which exceed 200 citations in several chapters. For the reviewing surgeon, good study questions may be more useful than innumerable references.

In its entirety *Vascular Surgery: A Comprehensive Review* is recommended as one excellent reference for the education of young general and vascular surgeons. It would also be one of my choices for preparing for the initial vascular board examination and for recertification.

JOHN W. HALLETT, JR.
Rochester, Minnesota

The History of Endocrine Surgery. Richard B. Welbourn, with contributions by Stanley R. Friesen, Ivan D. A. Johnston, and Ronald A. Sellwood. 409 pp. Westport, CT: Praeger, 1990. $75.00.

THIS BOOK BY Richard B. Welbourn, one of the world's most distinguished endocrine surgeons, will not disappoint those who were aware of its preparations and were eagerly awaiting its publication. Professor Welbourn, who until his retirement from London University's Royal Postgraduate Medical School at Hammersmith Hospital, was Britain's first and only designated professor of endocrine surgery. He is eminently qualified for the task of placing into perspective for the reader those events and discoveries that have led to the development of a new and expanding discipline within general surgery. He does so with a superb writing style, emphasizing contributions made by surgeons while nevertheless not neglecting those made by researchers in the basic sciences or in endocrinology. The author, who himself made many important contributions to endocrine surgery, quotes Billroth in his preface who said, 'Only the man who is familiar with the art and science of the past is competent to aid in its progress in the future.' This book was conceived with that purpose for all of those with interest in endocrinology and surgery of the endocrine glands, and details this fascinating story from its origins until about 1980.

After an introductory chapter on the evolution of endocrine surgery, the book is divided into major chapters on the thyroid, the pituitary, the adrenal glands, the parathyroid glands, and the endocrine gut and pancreas. Both Ivan D. A. Johnston and S. R. Friesen also contributed significantly to several of these chapters. Additional chapters include the history of the multiple endocrine adenopathy and paraendocrine syndromes, cancer of the breast and prostate, essential and renal hypertension, and surgical stress. Throughout the book is so well written and edited that it is difficult to put down. Two additional features of the book are a chronology beginning in antiquity and ending in 1982, outlining both important world events as well as related scientific discoveries important to the field of endocrine surgery and a biographical index, as well as portraits of surgeons and others who have made important contributions. If, as Thomas Carlyle stated, 'history is the biography of great men,' the reader will become familiar with all who were part of this story. Oliver Bearhs notes in his foreword to this book that Richard B. Welbourn has done a great service in bringing together in one publication the contributions of the giants of the past and the historical facts, many of which remain pertinent in the practice of endocrinology today. It is the foundation of our current knowledge and is necessary for our present education, research, and clinical practice. This is a book that all physicians would enjoy and profit from reading. It is a must for surgeons with an interest in endocrinology, and the same could equally apply to endocrinologists.

NORMAN W. THOMPSON, M.D.
Ann Arbor, Michigan

Current Practice of Surgical Stapling. Mark M. Ravitch, Felicien M. Steichen, and Roger Welter. 324 pp. Philadephia: Lea & Febiger, 1991. $79.50.

THE WORK *Current Practice of Surgical Stapling* was published as a result of the Second International Symposium and First European Congress on Stapling in Surgery, for which Drs. Ravitch and Steichen were Co-Presidents. This book could be reviewed on several levels. The first is that it represents one of the last efforts in academic surgery in the long and glorious career of Dr. Mark M. Ravitch and is a typically well thought out and beautifully executed work dealing with an area that clearly meant a great deal to him. Currently surgical stapling is an important part in training residents not only in gastrointestinal surgery but also (as noted in this book) in thoracic and colorectal surgery. I was not fortunate enough to know Dr. Ravitch personally; however many other areas of Dr. Ravitch's writings and scholarly pursuits are a matter of record and this appears to be the equal to any of them.

In terms of an informative and complete work on stapling, the book is a complete success. It is more than just a 'how to' or techniques manual. It explores the history and principles of stapling in surgery in a personal manner as described by Dr. Ravitch and Steichen in the first two chapters. As such many physiologic as well as 'biopolitical' aspects of the advent of stapling are described in a personal way as Dr. Ravitch initially encountered stapling in Russia in the late 1950s. Other chapters look at some of the older instruments and the development of the more modern instruments currently used in the operating room. The adjunctive use of biologic (fibrin) glue is explored. Other areas deal with comparison both clinically and in the laboratory with manual suture techniques. Newer considerations, such as the use of stapled devices in previously irradiated fields, are also addressed. The use of staplers specifically in gastric, pulmonary, and esophageal surgery are presented by internationally known experts from Europe, the United States, and Japan. A broad spectrum of indications, problems, and frank discussions of results are presented. There is very little left unsaid in this complete work, which serves as a valuable reference source for those interested in a complete analysis of widely used techniques of stapling in the United States today.

It is indeed sad to think that Dr. Ravitch has passed from the surgical scene. He was clearly a giant who left a large void with his passing. It is nice to think that one of his final efforts is a complete, thorough, and thoughtful analysis of one of the areas of clinical surgery that he loved so much, surgical stapling. This book should be a part of the surgical library of any teaching institution and its first author should be recognized as an academic giant whose career allowed us to enjoy significant advances in the operating room using technology, physiology, and sound surgical judgment.

JOHN B. HANKS, M.D.
Charlottesville, Virginia

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David C. Sabiston, Jr., M.D.
Annals of Surgery
Department of Surgery
Duke University Medical Center
Durham, North Carolina 27710

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Tables: each table, complete with title and footnotes, on a separate page
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Illustrations must be good quality, unmounted glossy prints, usually 12.7 by 17.3 cm (5 by 7 in.), but no larger than 20.3 by 25.4 cm (8 by 10 in.).

Submit the required number of copies of manuscript and figures (*see* journal's instructions) in heavy-paper envelope. Submitted manuscript should be accompanied by covering letter, as described in Submission of Manuscripts, and permissions to reproduce previously published materials or to use illustrations that may identify subjects.

Follow journal's instructions for transfer of copyright.

Authors should keep copies of everything submitted.

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Type manuscript on white bond paper, 20.3 by 26.7 cm or 21.6 by 27.9 cm (8 by 10½ in. or 8½ by 11 in.) or ISO A4 (212 by 297 mm) with margins of at least 2.5 cm (1 in.). Use double spacing throughout, including title page, abstract, text, acknowledgments, references, tables, and legends for illustrations. Begin each of the following sections on separate pages: title page, mini abstract, abstract, text, acknowledgments, references, individual tables, and legends. Number pages consecutively, beginning with the title page. Type the page number in the upper right-hand corner of each page.

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The title page should contain [1] the title of the article, which should be concise but informative; [2] a short running head or footline of no more than 40 characters (counting letters and spaces) placed at the foot of the title page and identified; [3] first name, middle initial, and last name of each author, with highest academic degree(s); [4] name of department(s) and institution(s) to which the work should be attributed; [5] disclaimers, if any; [6] name and address of author responsible for correspondence about the manuscript; [7] name and address of author to whom requests for reprints should be addressed, or statement that reprints will not be available from the author; [8] the source(s) of support in the form of grants, equipment, drugs, or all of these.

MINI ABSTRACT

A short description of the study should be submitted to appear on the table of contents. It should consist of approximately three sentences of no more than 50 words.

ABSTRACT

The second page should carry an abstract of not more than 150 words. The abstract should state the purposes of the study or investigation, basic procedures (study subjects or experimental animals and observational and analytic methods), main findings (give specific data and their statistical significance, if possible), and the principal conclusions. Emphasize new and important aspects of the study or observations. Use only approved abbreviations (*see* list of Commonly Used Approved Abbreviations elsewhere in this document).

TEXT

The text of observational and experimental articles is usually, but not necessarily, divided into sections with the headings Introduction, Methods, Results, and Discussion. Long articles may need subheadings within some sections to clarify their content, especially the Results and Discussion sections. Other types of articles such as case reports, reviews, and editorials may need other formats, and authors should consult individual journals for further guidance.

Introduction: Clearly state the *purpose* of the article. Summarize the rationale for the study or observation. Give only strictly pertinent references, and do not review the subject extensively.

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When reporting experiments on human subjects, indicate whether the procedures followed were in accord with the ethical standards of the Committee on Human Experimentation of the institution in which the experiments were done or in accord with the Helsinki Declaration of 1975. When reporting experiments on animal subjects, indicate whether the institution's or the National Research Council's guide for the care and use of laboratory animals was followed. Identify precisely all drugs and chemicals used, including generic name(s), dosage(s), and route(s) of administration. Do not use patients' names, initials, or hospital numbers.

Include numbers of observations and the statistical significance of the findings when appropriate. Detailed statistical analyses, mathematical derivations, and the like sometimes may be suitably presented in the form of one or more appendixes.

Results: Present your results in logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables and/or illustrations: emphasize or summarize only important observations.

Discussion: Emphasize the new and important aspects of the study and *conclusions* that follow from them. Do not repeat in detail data given in the Results section. Include in the Discussion the implications of the findings and their limitations and relate the observations to other relevant studies. Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such. Recommendations, when appropriate, may be included.

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The references must be verified by the author(s) against the original documents.

Examples of correct forms of references are given below.

Journal

1. Standard Journal Article (List all authors when six or less; when seven or more, list only first three and add et al.)

   Soter NA, Wasserman SI, Austen KF. Cold urticaria: release into the circulation of histamine and eosinophil chemotactic factor of anaphylaxis during cold challenge. N Engl J Med 1976; 294:687–690.

2. Corporate Author

   The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended method for the determination of gammaglutamyltransferase in blood. Scand J Clin Lab Invest 1976; 36:119–125.

   Anonymous. Epidemiology for primary health care. Int J Epidemiol 1976; 5:224–225.

Books and Other Monographs

3. Personal Author(s)

   Osler AG. Complement: mechanisms and functions. Englewood Cliffs: Prentice-Hall, 1976.

4. Corporate Author

   American Medical Association Department of Drugs. AMA drug evaluations. 3rd ed. Littleton: Publishing Sciences Group, 1977.

5. Editor, Compiler, Chairman as Author

   Rhodes AJ, Van Rooyen CE, comps. Textbook of virology: for students and practitioners of medicine and the other health sciences. 5th ed. Baltimore: Williams & Wilkins, 1968.

6. Chapter in Book

   Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: WB Saunders, 1974:457–471.

7. Agency Publication

   National Center for Health Statistics. Acute conditions: incidence and associated disability, United States July 1968–June 1969. Rockville, Md.: National Center for Health Statistics, 1972. (Vital and health statistics. Series 10: Data from the National Health Survey, no. 69) (DHEW publication no. (HSM)72-1036).

Other Articles

8. Newspaper Article

   Shaffer RA. Advances in chemistry are starting to unlock mysteries of the brain: discoveries could help cure alcoholism and insomnia, explain mental illness. How the messengers work. Wall Street Journal 1977 Aug 12:1(col. 1), 10(col. 1).

9. Magazine Article

   Roueché B. Annals of medicine: the Santa Claus culture. The New Yorker 1971 Sep 4:66–81.

TABLES

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Omit internal horizontal and vertical rules.

Cite each table in the text in consecutive order.

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ABBREVIATIONS

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In most countries the International System of Units (SI) is standard or is becoming so. Report measurements in the units in which they were made. Journals may use these units, convert them to another system, or use both.

COMMONLY USED APPROVED ABBREVIATIONS

Standard Units of Measurement

Term	Abbreviation or Symbol
ampere	A
ångström	Å
barn	b
candela	cd
coulomb	C
counts per minute	cpm
counts per second	cps
curie	Ci
degree Celsius	°C
disintegration per minute	dpm
disintegration per second	dps
electron Volt	eV
equivalent	Eq
farad	F
gauss	G
gram	g
henry	H
hertz	Hz
hour	h
international unit	IU
joule	J
kelvin	K
kilogram	kg
liter	L
meter, metre	m
minute	min
molar	mol/L
mole	mol
newton	N
normal (concentration)	N
ohm	Ω
osmol	osmol
pascal	Pa
revolutions per minute	rpm
second	s
square centimeter	cm^2
volt	V
watt	W
week	wk
year	yr

Combining Prefixes

tera-	(10^{12})	T
giga-	(10^{9})	G
mega-	(10^{6})	M
kilo-	(10^{3})	k
hecto-	(10^{2})	h
deca-	(10^{1})	da
deci-	(10^{-1})	d
centi-	(10^{-2})	c
milli-	(10^{-3})	m
micro-	(10^{-6})	μ
nano-	(10^{-9})	n
pico-	(10^{-12})	p
femto-	(10^{-15})	f
atto-	(10^{-18})	a

Statistical Terms

correlation coefficient	r
degrees of freedom	df
mean	$\bar{x}$
not significant	NS

number of observations	n
probability	p
standard deviation	SD
standard error of the mean	SEM
"Student's" t test	t test
variance ratio	F

Others

adenosinediphosphatase	ADPase
adenosine 5′-diphosphate (adenosine diphosphate)	ADP
adenosine 5′-monophosphate (adenosine monophosphate, adenylic acid)	AMP
adenosine triphosphatase	ATPase
adenosine 5′-triphosphate (adenosine triphosphate)	ATP
adrenocorticotropic hormone (adrenocorticotropin)	ACTH
bacille Calmette-Guérin	BCG
basal metabolic rate	BMR
body temperature, pressure, and saturated	BTPS
central nervous system	CNS
coenzyme A	coA
deoxyribonucleic acid (deoxyribonucleate)	DNA
dihydroxyphenethylamine	dopamine
electrocardiogram	ECG
electroencephalogram	EEG
enteric cytopathogenic human orphan (virus)	ECHO
ethyl	Et
ethylenediaminetetraacetate	EDTA
gas-liquid chromatography	GLC
guanosine 5′-monophosphate (guanosine monophosphate, guanylic acid)	GMP
hemoglobin	Hb
logarithm (to base 10; common logarithm)	log
logarithm, natural	ln
methyl	Me
Michaelis constant	K_m
negative logarithm of hydrogen ion activity	pH
partial pressure of CO_2	P_{CO_2}
partial pressure of O_2	P_{O_2}
per	/
percent	%
radiation (ionizing, absorbed dose)	rad
respiratory quotient	RQ
specific gravity	sp gr
standard atmosphere	atm
standard temperature and pressure	STP
ultraviolet	uv
volume	vol
volume ratio (volume per volume)	vol/vol
weight	wt
weight per volume	wt/vol
weight ratio (weight per weight)	wt/wt

ABBREVIATIONS OF NAMES OF FREQUENTLY CITED JOURNALS

Acta Medica Scandinavica	Acta Med Scand
American Family Physician	Am Fam Physician
American Heart Journal	Am Heart J
American Journal of Cardiology	Am J Cardiol
American Journal of Clinical Nutrition	Am J Clin Nutr
American Journal of Clinical Pathology	Am J Clin Pathol
American Journal of Digestive Diseases	Am J Dig Dis
American Journal of Diseases of Children	Am J Dis Child
American Journal of Human Genetics	Am J Hum Genet
American Journal of the Medical Sciences	Am J Med Sci
American Journal of Medicine	Am J Med
American Journal of Obstetrics and Gynecology	Am J Obstet Gynecol
American Journal of Ophthalmology	Am J Ophthalmol
American Journal of Pathology	Am J Pathol
American Journal of Physical Medicine	Am J Phys Med
American Journal of Physiology	Am J Physiol
American Journal of Psychiatry	Am J Psychiatry
American Journal of Public Health	Am J Public Health
American Journal of Roentgenology	AJR
American Journal of Surgery	Am J Surg
American Journal of Tropical Medicine and Hygiene	Am J Trop Med Hyg
American Review of Respiratory Disease	Am Rev Respir Dis
Anaesthesia	Anaesthesia
Anesthesiology	Anesthesiology
Annals of Allergy	Ann Allergy
Annals of Internal Medicine	Ann Intern Med
Annals of Otology, Rhinology and Laryngology	Ann Otol Rhinol Laryngol
Annals of Surgery	Ann Surg
Annals of Thoracic Surgery	Ann Thorac Surg
Archives of Dermatology	Arch Dermatol
Archives of Environmental Health	Arch Environ Health
Archives of General Psychiatry	Arch Gen Psychiatry
Archives of Internal Medicine	Arch Intern Med
Archives of Neurology	Arch Neurol
Archives of Ophthalmology	Arch Ophthalmol
Archives of Otolaryngology	Arch Otolaryngol
Archives of Pathology and Laboratory Medicine	Arch Pathol Lab Med
Archives of Physical Medicine and Rehabilitation	Arch Phys Med Rehabil
Archives of Surgery	Arch Surg
Arthritis and Rheumatism	Arthritis Rheum
Blood; Journal of Hematology	Blood
Brain; Journal of Neurology	Brain
British Heart Journal	Br Heart J
British Journal of Obstetrics and Gynaecology	Br J Obstet Gynaecol
British Journal of Radiology	Br J Radiol
British Journal of Surgery	Br J Surg
British Medical Journal	Br Med J
Canadian Journal of Public Health	Can J Public Health
Canadian Medical Association Journal	Can Med Assoc J
Cancer	Cancer
Chest	Chest
Circulation; Journal of the American Heart Association	Circulation
Circulation Research	Circ Res
Clinical Pediatrics	Clin Pediatr (Phila)
Clinical Pharmacology and Therapeutics	Clin Pharmacol Ther
Clinical Science and Molecular Medicine	Clin Sci Mol Med
Clinical Toxicology	Clin Toxicol
Diabetes	Diabetes
DM, Disease-a-Month	DM
Endocrinology	Endocrinology
Gastroenterology	Gastroenterology
Geriatrics	Geriatrics
Gut	Gut
Human Pathology	Hum Pathol
Investigative Radiology	Invest Radiol
JAMA; Journal of the American Medical Association	JAMA
Journal of Allergy and Clinical Immunology	J Allergy Clin Immunol
Journal of Applied Physiology	J Appl Physiol
Journal of Biological Chemistry	J Biol Chem
Journal of Bone and Joint Surgery; American Volume	J Bone Joint Surg [Am]
Journal of Bone and Joint Surgery; British Volume	J Bone Joint Surg [Br]
Journal of Clinical Endocrinology and Metabolism	J Clin Endocrinol Metab
Journal of Clinical Investigation	J Clin Invest
Journal of Clinical Pathology	J Clin Pathol
Journal of Experimental Medicine	J Exp Med
Journal of Gerontology	J Gerontol
Journal of Immunology	J Immunol
Journal of Infectious Diseases	J Infect Dis
Journal of Investigative Dermatology	J Invest Dermatol
Journal of Laboratory and Clinical Medicine	J Lab Clin Med
Journal of Laryngology and Otology	J Laryngol Otol
Journal of Medical Education	J Med Educ
Journal of Nervous and Mental Disease	J Nerv Ment Dis
Journal of Neurosurgery	J Neurosurg
Journal of Pathology	J Pathol
Journal of Pediatrics	J Pediatr
Journal of Physiology	J Physiol
Journal of Thoracic and Cardiovascular Surgery	J Thorac Cardiovasc Surg
Journal of Trauma	J Trauma
Journal of Urology	J Urol
Lancet	Lancet
Medical Clinics of North America	Med Clin North Am
Medical Letter on Drugs and Therapeutics	Med Lett Drugs Ther
Medicine (Baltimore)	Medicine (Baltimore)
New England Journal of Medicine	N Engl J Med
Obstetrics and Gynecology	Obstet Gynecol
Pediatrics Clinics of North America	Pediatr Clin North Am
Pediatrics	Pediatrics
Physiological Reviews	Physiol Rev
Plastic and Reconstructive Surgery	Plast Reconstr Surg
Postgraduate Medicine	Postgrad Med
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Public Health Reports	Public Health Rep
Radiology	Radiology
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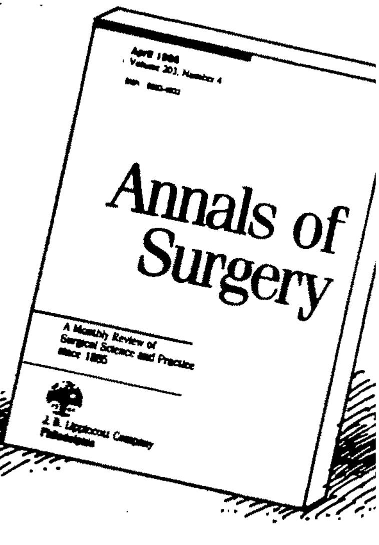
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Rejection Revisited

The 1990 Nobel Prize in Medicine was presented to Dr. Joseph Murray of Boston for his pioneering work in organ transplantation. In the 1950s, when Dr. Murray was performing the first successful human renal transplants, transplant rejection was poorly understood. The prevailing scientific beliefs suggested that all allografts would fail. The new basic science of transplantation immunology was inadequate to explain the seemingly miraculous events that occurred in the early years of clinical organ transplantation. In fact the success of human organ transplantation challenged the basic scientists to provide a more adequate understanding and thus created one of the best examples of successful collaboration between clinical and basic researchers. Early investigators studied the time course and histopathology of allograft rejection, from which the initial concepts of humoral and cellular mechanisms were derived. Modern molecular biology has provided techniques that vastly expand our ability to understand the allograft reaction at cellular and subcellular levels.

It is fitting that rejection is now revisited by two Boston colleagues of Dr. Murray, Drs. Nicholas L. Tilney and Jerzy W. Kupiec-Weglinski, who themselves illustrate well the power of collaboration between thoughtful basic scientists and innovative clinical surgeons. In this issue of the *Annals of Surgery*, they provide a clear and compelling update on the biology of acute transplant rejection.[1]

This concise What's New in General Surgery article contains a wealth of information on the several types of cells involved in transplant rejection, both as stimulators of the immune response and effectors of graft destruction. Current concepts of antigen presentation and recognition, effector function, and host regulatory mechanisms are lucidly presented. Just as clearly, current knowledge is incomplete and inadequate for routine induction of transplantation tolerance.

New immunosuppressive agents such as FK506 (Fujisawa Pharmaceutical, Osaka, Japan) and Rapamycin (Wyeth-Ayerst, Princeton, NJ), which are presently the subjects of clinical and experimental animal transplantation trials, respectively, are up to 1000 times more potent on a milligrams per kilogram basis than the agents now in clinical use. Although there exists no clear understanding of their mechanisms of action, they are likely to maintain the momentum of the transplant revolution in surgery. If empiric clinical data support the effectiveness of these pharmacologic agents, just as was the case more than a quarter century ago, basic scientists will hasten to elucidate the mechanisms that underlie the successful clinical results. The situation will only change when our detailed knowledge of acute allograft rejection at the molecular level permits prospective intervention at precisely the right time and place to interrupt specifically the destructive immune cascade. Drs. Tilney and Kupiec-Weglinski have fixed our present position along the path to that high level of comprehension. Continuing cooperation between basic and clinical scientists should produce an ever more molecular understanding that suggests specific interventions to blunt allograft rejection. One should not be surprised, however, if surgical scientists are still leading the way when rejection is revisited a quarter century from now.

R. RANDAL BOLLINGER, M.D.
Durham, North Carolina

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WHAT'S NEW IN GENERAL SURGERY

The Biology of Acute Transplant Rejection

NICHOLAS L. TILNEY, M.D., and JERZY W. KUPIEC-WEGLINSKI, PH.D., M.D.

An intriguing and increasingly understood facet of immune responses is the ability of a recipient to destroy a foreign tissue or organ graft. The phenomenon of acute rejection of an allograft involves a series of complex and inter-related cellular and humoral events, culminating in graft death. Some of the current thinking surrounding this phenomenon is reviewed.

From the Surgical Research Laboratory, Harvard Medical School and the Department of Surgery, Brigham & Women's Hospital, Boston, Massachusetts

THE IMMUNE SYSTEM has evolved from primitive beginnings in lower organisms to one of remarkable diversity and sophistication in mammals. Its function is to protect the host throughout its lifetime by inactivating or destroying foreign antigens, presumably including mutant host cells bearing potentially malignant characteristics. Because the realization that this critical aspect of the host defense mechanisms is both monitored and mediated primarily by lymphoid cells, increasing interest among biologists has become centered on lymphocytes, their behavior and function, their interactions, and their products. These cells are ubiquitous throughout the body, make up approximately 20% of circulating leukocytes, and are concentrated in thoracic duct lymph, bone marrow, submucosa of the gut, spleen, lymph nodes, thymus, tonsils, and Peyer's patches. Overall the lymphoid tissues comprise an organ of considerable size.

Because the phenomenon of rejection has become recognized as a lymphocyte-directed immunologic event, investigations into this dramatic, complex, and powerful panoply of host defenses brought into play by the stimulus of an allograft and leading to its destruction have increased dramatically during the past few decades. Indeed definition of these responses, sharpened by coincident advances in several related biosciences, which include immunogenetics, cell biology, molecular biology and pharmacology, have led to an explosion in knowledge undreamed of by the original pioneers in these fields.

The modern era of transplantation biology was opened by Medawar during World War II with his observations with Gibson on the differences in behavior between skin autografts and allografts placed on severely burned patients. In subsequent experimental studies in rabbits, he described a progressive infiltration of the graft and graft bed by host mononuclear cells leading to its destruction about 1 week after placement.[1] He also found that when additional skin from the same donor was later transplanted to these animals, rejection of the 'second set' grafts occurred more rapidly than the initial event. The immunologic nature of these phenomenon were reinforced by dramatic changes in the lymph nodes draining orthotopic skin allografts, which included the appearance of large numbers of lymphoid cells in cortical nodules and marked proliferation of plasma cells in medullary cords.[2] The coincident development of genetically defined inbred mouse strains by Snell and others[3] provided the opportunity to use reproducible animal models in the burgeoning biology of transplantation immunology. Using such inbred strains, Mitchison[4] and Billingham, Brent, and Medawar[5] conferred immune reactivity to otherwise naive mice by adoptive transfer of lymphocytes from animals of the same strain that had previously responded to a particular tumor or skin graft; as such immunity could not be transferred consistently with serum, antibody responses were considered of secondary importance in the acute destruction of foreign tissues. Gowans[6,7] then showed that many small lymphocytes recirculated continuously in the body and were 'immunologically competent.' Subsequently histocompatibility antigens, defined at cellular and molecular levels, were found both to stimulate and provide targets for host immune responsiveness. More recently, rapid advancements in hybridoma technology with increasing availability of monoclonal antibodies (MAb) directed specifically against individual antigenic determinants are allowing more complete char-

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Address reprint requests to Nicholas L. Tilney, M.D., Brigham & Women's Hospital, 75 Francis St., Boston, MA 02115.

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acterization of the cellular cascade mediating rejection, as well as progressive understanding of the actions, interrelationships, influences and contributions of cell populations, subpopulations, and their factors in this extraordinary host event.

Based on the experience in clinical transplantation during the last three decades, patterns of the host immune responses against foreign organs or tissues have become appreciated, each with its relatively discreet histopathology, host mechanisms of destruction, timing, and prognosis. Hyperacute rejection is considered primarily a humoral event manifested by rapid interaction between cytotoxic antibodies circulating in the presensitized recipient and graft antigen expressed predominantly on its vascular endothelium. Inflammatory mediators including those of the complement, kinin, and coagulation systems are activated and released, resulting in vascular dissolution, interstitial hemorrhage, microvascular thrombosis, and graft death. Accelerated rejection includes both humoral and cellular components of host immunity and occurs within a few days of engraftment in recipients previously sensitized against donor antigens. Acute rejection is primarily a cellular (T lymphocyte mediated) phenomenon that appears 1 week or later in desensitized hosts. Chronic rejection implies gradual fibrosis of the graft that is manifested by declining function over months or years, and is thought to be predominantly on a humoral basis. As most is known currently about its biology, this review will be limited to the events of acute rejection, although it should be stressed that the above conditions are not discrete entities unto themselves but are undoubtedly part of a spectrum or continuum of host immune activity.

The Cells Responsible for Acute Rejection

Progressive infiltration of the graft substance by host mononuclear cells is characteristic of acute rejection of allogeneic tissues of all types. Within a few hours of revascularization, a few small host lymphocytes infiltrate perivascular areas and then scatter throughout the graft substance. As the inflammatory reaction proceeds, macrophages became more obvious, with disruption of pericapillary tissues, progressive interstitial inflammation, and eventual tissue necrosis. At the same time, numbers of lymphocytic cells, both mature and blastic, increase expedientially in recipient lymphoid compartments.

The host cells entering rejecting allografts and migrating to lymphoid tissues include T and B lymphocytes, macrophages, and natural killer (NK) cells.[1,8,9] The primary responsibility of T cells in the acute destructive process has been emphasized *in vitro* in the lymphocyte-mediated cytoxicity assay by their ability to lyse donor target cells by direct interaction without the need for antibody,[10] and *in vivo* by the inability of T-cell–depleted animals to reject allografted tissues.[11] The increasing availability of specific MAbs also has allowed fractionation of T lymphocytes into their subpopulations, cytotoxic/suppressor (Tc/s) cells, and T helper/inducer (Th) phenotype.[12] The current phenotypic nomenclature for Tc/s and Th lymphocytes is CD8 and CD4, respectively; CD8 is comparable to OKT8 or LEU-2, LYT-2 and OX8 in human, mouse and rat, respectively, while CD4 is comparable to OKT4 or LEU-3, or L3T4, and W3/25, respectively. It should be emphasized, however, that identification of surface antigens expressed on resting Tc/s and Th cells wrongly suggests a direct correlation between phenotype and function; indeed particular states of host immune responsiveness may change cell relationships and alter phenotype. Thus it has become apparent that the use of MAbs directed against activation markers such as specific receptors developing on the surfaces of antigen-activated cells has allowed better understanding of function of various cell subpopulations than their identification by phenotype alone.[13] The sharing of a phenotype by Tc/s also presents a conceptual problem because these populations or a single Tc/s population may either destroy allogeneic cells or suppress their alloaggressive functions, behavior that is presumably dependent on particular conditions of host activation.

In addition to T lymphocytes, other cell populations infiltrate rejecting grafts. B lymphocytes, when allostimulated, differentiate into antibody-producing plasma cells; large numbers of immunoblasts and plasma cells are seen in host spleen within a few days of engraftment. B cells from the graft infiltrate secrete both nonspecific antibodies and those specific against the donor.[14] However, despite their obvious activity, their actual role in acute cell-mediated rejection remains enigmatic and not well defined. Natural killer cells, a population of cytotoxic, non-T,non-B cells, destroy certain tumor cells *in vitro*; although they infiltrate organ allografts rapidly, deletion experiments in graft recipients using specific anti-NK cell antibodies show that they are not critical in the acute rejection process.[15] In antigen–antibody interactions, however, where the constant Fc portion of the antibody is exposed, NK cells adhere to the Fc molecules and presumably contribute to the rejection process.

Macrophages act both as antigen-presenting cells that initiate immune responsiveness and as alloaggressive cells that contribute to graft destruction.[16] Their unrelated roles in the host defenses are several. (1) Macrophages may carry or process antigen activated lymphocyte populations by direct contact. (2) They may act as the principle site of control by immune response genes. (3) Receptors on their plasma membranes may cause destruction of antibody-coated target cells, activation of B cells by antigen, and location of antigen–antibody complexes. (4) They may elaborate cytokines when activated, particularly interleukin-1 (IL-1), which in turn activates CD4+ lymphocytes. Lymphokines released by activated CD4+ cells, particularly gamma interferon (IFNγ), may stimulate naive macrophages to become alloaggressive against the graft.

Mechanisms of Acute Allograft Rejection

Like a neurologic arc, the immunologic host responses to foreign tissues have been conceptualized as comprising two limbs, an afferent or sensitizing limb and an efferent or effector limb.[7] Following interactions between circulating host lymphocytes and graft antigen or antigen-presenting cells, specifically sensitized effector cells enter the graft substance *via* its vasculature and presumably cause or at least trigger its destruction by attracting large numbers of nonspecific lymphocytes and macrophages to its substance. Other sensitized cells proliferate in host lymphoid tissues. The actual biologic mechanisms by which these events occur are gradually becoming unravelled.

Graft Immunogenicity

Although not well defined, cellular elements within the transplanted tissues themselves may possess antigenic determinants of varying immunostimulatory capacities. Epidermal cells, vascular endothelium, donor leukocytes, or dendritic cells (DC) isolated from the graft substance, spleen, or afferent lymph may cause histoincompatible allogeneic leukocytes to proliferate vigorously *in vitro*; *in vivo* transfer of such cells can elicit antibody production by the host or induce rejection of established organ allografts following transfer.[17,18] 'Passenger leukocytes' residing within the graft have been thought important in host sensitization. Removal of DCs from mouse pancreatic islets before transplantation, for instance, renders the islets nonimmunogenic, while administration of DCs before engraftment may cause rejection of tissues transplanted thereafter.[19] Such findings are not uniform, however, because heart grafts are rejected in an accelerated fashion in some mouse strains that had received DCs before transplantation but not in those that were given previous blood transfusions.[20]

There may be several subpopulations of DCs. Langerhans cells in skin are derived from bone marrow. Their reduction by donor pretreatment with x radiation, corticosteroids, or other leukocytic agents increase skin graft survival.[17] These cells migrate from blood to spleen, enter T-cell–dependent areas from the splenic marginal zone, and interact closely with T-cell subpopulations, primarily Th.[21] Not only can they present antigen to sensitized T cells but they can activate resting T cells. Lymphoid DCs, isolated from spleen, are immunostimulatory to T cells and have characteristic phenotype but lack markers for macrophages and lymphocytes. They are widely scattered throughout many tissues in the body, both lymphoid and nonlymphoid, and migrate in blood and lymph. In addition DCs from grafted tissues may migrate to spleen,[22] an observation at variance with the usual dogma that suggests that sensitization of host cells occurs within the graft itself and not in host lymphoid tissues. Migration of DCs from tissue to tissue *via* the blood stream and lymph seems to be a physiologic process that may amplify sensitization of the host to an antigen.

Tissue-specific antigens also may be important components of immunogenicity; transplants of pure epidermal cells from skin bearing putative 'skin specific antigens' are rejected acutely, while different antigen systems in organ allografts may initiate their rejection.

The immunogenic variation between skin and primarily vascularized allografts also has been explained on the basis of the route of host sensitization. Unlike organ grafts, which are revascularized promptly, skin grafts are laid directly on the recipient dermal bed. Complete revascularization apparently is not necessary for immunostimulation because a 3- to 4-day exposure to a first-set skin graft will sensitize the host; the more rapid re-establishment of lymphatic drainage within that period allows percolation of particulate graft antigen from skin to regional lymph nodes, a more important route of sensitization in this context than migration of passenger leukocytes to recipient lymphoid tissues or host lymphocytes circulating to the graft.[23] The importance of lymphatics in host sensitization in particular anatomic locations has been emphasized by the relative protection conferred on tissue allografts placed in 'immunologically privileged' sites, which include the anterior chamber of the eye, brain, hamster cheek pouch, and vascularized skin pedicles with surgically interrupted lymphatics.[24]

In contrast direct revascularization of organ allografts is critical for host immunization; the tempo of rejection is unaffected by isolation of kidney grafts from recipient lymphatics by placing the organ in a plastic bag.[25] Nor is the rejection rate influenced when efferent lymph from the graft is diverted.[26] Circulating host effector cells become sensitized by contact with the antigens of a primarily revascularized graft. Many cells may migrate into the organ; those specifically sensitized to graft antigen may be retained selectively within it and in turn may attract and mobilize large numbers of uncommitted potentially alloaggressive cells by direct interaction. That only a small population of specifically sensitized cells is necessary to trigger the effector responses has been shown by radiolabeling experiments tracing lymphocytes sensitized against different antigens; there is always selective infiltration of small but critical numbers of antigen-specific cells into the appropriate allograft.[27]

Antigen Presentation and Recognition

Antigens on allogeneic cell surfaces allow the host to recognize that the transplanted tissue is not 'self.' It has been long known that the immune responses between genetically dissimilar humans are directed against a single cluster of alloantigens, designated human leukocyte antigen (HLA) and encoded by major histocompatibility complex (MHC) genes found on chromosome six.[28] There are two MHC antigen groups: class I (HLA-A and B) and class II (HLA-D and DR), both prime targets for host immunoreactivity. These are expressed characteristically on various mammalian tissues, may be up or down regulated by host cells and their products, and may serve to trigger graft rejection differentially. Class I antigens are relatively ubiquitous and are constituitively expressed throughout somatic cells; these interact exclusively with and activate CD8+ T lymphocytes. Class II antigens are distributed more selectively throughout lymphoid tissues, on DCs, Langerhans cells in skin, circulating B lymphocytes, and monocytes. Their distribution on vascular endothelial cells is a particularly important site for immune injury because endothelium is exposed continuously to circulating effector cells and their products. CD4+ T lymphocytes are activated selectively by class II antigens. In addition cell mediators or lymphokines can regulate expression of MHC antigens selectively by inducing different class II gene products or promoting class I antigens differentially over class II. Interferon γ, for instance, can upregulate class II antigen on several cell types, including lymphocytes, pancreatic beta cells, vascular endothelium, and renal tubular cells; this upregulation increases antigen presentation and amplifies graft immunogenicity.[29] Transplanted tissues or their components also may differ in expression of these antigen; in the rat, class I expression develops more rapidly in the allografted kidney than in the heart, increasing many times (40x) within a few days of transplantation. In contrast the kinetics of class II induction is similar in kidney and heart grafts.[30]

Recognition of graft 'foreigness' by T lymphocytes requires both the MHC molecule and the alloantigen.[31] The spatial configuration of this event involves interaction between the antigen receptor of specific T cells and a foreign peptide bound to a groove in the MHC molecule; this complex arrangement allows presentation of alloantigen to the T-cell receptor *via* its alpha and beta cell-surface molecules.[32] The immunologic function of the remainder of the T-cell antigen receptor (gamma, delta, and zeta chains) is not clear. Genetic rearrangements of alpha and beta genes, covalently linked by disulfide bonds, produce combining sites on the cell surface that can recognize virtually any antigen. The phenotypic structures CD4 and CD8 also act as accessory molecules to increase the avidity of the interaction between the MHC antigen and the T-cell receptor. Once specific alloantigens have been recognized, the T lymphocyte becomes activated, a process involving complex intracellular changes that result in clonal expansion, with differentiation and proliferation of a new generation of antigen-specific T cells. Antigen activation of CD4+ Th cells causes production and release of cell products and expression of various surface receptors. At the same time, activated CD8+ cells develop receptors for interleukin-2 (IL-2) and IL-2 receptor (IL-2R).

Antigen recognition by T lymphocytes alone is not thought to be sufficient to trigger host events. A two-signal process of lymphocyte activation has been suggested, involving binding of transplantation antigens to T-cell surface receptors (signal 1), which coincidentally receive a costimulating signal 2 from antigen-presenting cells.[33] Activation and differentiation of CD8+ T cells occurs after both signals but not by interaction with serologically defined antigens on the surfaces of allogeneic cells. CD8+ precursors, activated by class I antigens, differentiate and proliferate further after interaction with IL-2 produced by CD4+ lymphocytes, themselves activated both by class II antigens and IL-1.[8,34]

Responses in Host Lymphoid Tissue

The most marked histologic changes occurring after placement of orthotopic skin allografts take place in regional draining lymph nodes following the rather prompt (about 3 days) reconstitution of lymphatic drainage. In contrast, in recipients of vascularized organ grafts, the spleen responds most dramatically.[35] Within 2 days of engraftment, large pyroninophilic immunoblasts proliferate in the peripheral periarterial lymphocyte sheaths then migrate into the red pulp where they differentiate into plasma cells. These antibody-producing cells, which triple the volume of the red pulp by the time of actual graft rejection, gradually decrease in number thereafter and are replaced by immunoperoxidase-negative large and small lymphocytes, changes associated with a rapid antibody response occurring 3 to 5 days after transplantation and peaking by 7 days.

Lymphocyte migration studies have emphasized the importance of massive shifts in cell populations to host lymphoid and nonlymphoid tissues following the antigenic stimulus of an organ graft. Under physiologic conditions T lymphocytes recirculate continuously between blood, lymphoid tissues, and lymph.[6] In the event of an antigenic stimulus in the gut, from a site of inflammation in skin such as a delayed-type hypersensitivity (DTH) reaction, or from placement of an allograft, such migration patterns change drastically to allow the antigenic message to disseminate as widely and promptly as possible throughout the host immune system.[36] The spleen is an important point of interaction between host lymphocytes and graft antigen brought by antigen-presenting cells, or with lymphokines released into the circulation by antigen-activated cells. It also acts as a site for antigen recognition and activation by naive host cells. Under physiologic conditions normal splenocytes localize relatively selectively in spleen and away from mesenteric and peripheral lymph nodes; during acute rejection, however, marked homing of sensitized cells occur in lymph nodes and Peyers patches of the gut, presumably to disperse the antigenic message more generally throughout host lymphoid tissues. In contrast the lymphoid cells that accumulate in spleen during the early phases of the acute rejection process diminish rapidly thereafter because of apparent migration to the graft site.

At least some of the rapid changes in lymphocyte migration patterns after an antigenic stimulus can be explained by the development of adhesion molecules both on antigenic cell surfaces and on lymphocyte membranes.[37] As noted CD4 and CD8 molecules increase the avidity of the interaction between the MHC graft antigen and the T-cell receptor. In addition other adhesion molecules, distinct from antigen-specific receptors, may influence homing patterns of lymphocytes.[38–40] Leukocyte function-associated antigen (LFA-1) causes lymphocytes to bind to high endothelial venules in lymph nodes; once there they migrate between the endothelial cells and enter the nodal substance, where they pursue their immunologic function. Other adhesion molecules on endothelial surfaces, endothelial-leukocyte adhesion molecules or intracellular adhesion molecules, for instance, may alter physiologic migration patterns of recirculating lymphocytes and increase the immunologic message to the entire host. One can visualize how a single antigenic site can engender systemic immune responsiveness in an effective and rapid fashion.

The Efferent Limb

Understanding of the actual means by which sensitized host cells actually destroy a graft remains elusive. The current consensus on these events have evolved through study of serial histologic changes within the rejecting graft, identification by phenotype and assessment of function of cell populations and subpopulations entering the graft from the circulation, and appreciation of the role of humoral destructive factors.[8,41,42] In addition, once the rejection events are well underway, regulatory host mechanisms are initiated whereby these protean and powerful alloaggressive responses are tempered, attenuated and reversed to restore the host to immunologic homeostasis.

The Cells Involved

Acute rejection is primarily a T-lymphocyte–mediated phenomenon; the survival of chicken feathers transplanted to athymic T-cell–deprived nude mice is a compelling affirmation of this observation.[43] Until recently it was assumed that the CD8+ cytotoxic T lymphocyte was the critical effector element in the process because of its ability to lyse donor target cells *in vitro*.[10] However with the appreciation that CD4+ and CD8+ T cells could be stimulated differentially by class II and class I MHC molecules, respectively, has come the realization that CD4+ cells may activate other lymphocyte populations, primarily through elaboration of IL-2.[44] Although the seminal role of these cells in DTH responses in activating and mobilizing macrophages to the site of inflammation is well recognized,[34] both CD4+ and CD8+ T-lymphocyte subpopulations are necessary to produce rejection in transplantation models (Fig. 1). Such interaction has been shown most obviously by reconstitution experiments in immunodeficient hosts; T-cell subpopulations, alone or in combination and with or without the addition of IL-2, were adoptively transferred into T-cell–depleted animals that were otherwise unable to reject their organ allografts.[11,45] Acute rejection could only be recreated by reconstitution with all T-lymphocyte elements and IL-2.

The nature of the graft, the degree of host sensitization, and histocompatibility differences between donor and recipient also may influence the relative contribution of the T-cell subsets. Current thinking emphasizes CD4+ T helper cells to be the primary, initiating, and organizing component of immune responsiveness against allografted tissues, with the Tc/s CD8+ subpopulation recruited secondarily to the site to complete the acute rejection process.[8,41,42,44] In addition to the activity of alloaggressive macrophages, the cytotoxic effects of this latter subpopulation presumably destroy allogeneic tissue directly. Following rejection both T-cell subsets may then revert to a resting state as memory cells. In contrast both CD4+ and CD8+ subpopulations rapidly mediate the accelerated rejection of a second donor strain transplant.[46] Overall it appears that graft rejection reflects different contributions of not one but multiple effector mechanisms.

Cell Products and Their Receptors

With definition of the cell populations involved in the immunologic host responses against allografted tissues has come appreciation of the critical importance of their products, the cytokines and lymphokines (Fig. 2). Various effector cell populations may produce one or more factors that, in turn, activate (or suppress) the function of other cell subpopulations. As noted antigen-activated macrophages elaborate IL-1, a monokine that stimulates CD4+

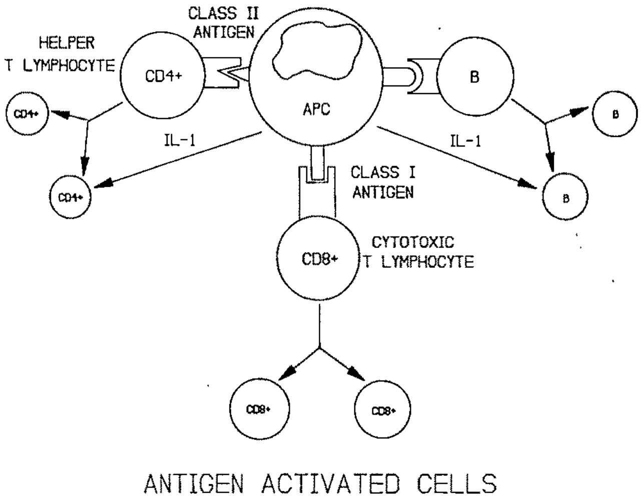


FIG. 1. Various lymphocyte populations are activated directly by graft antigen or by antigen-processing cells (APC) expressing MHC antigens. IL-1, a cytokine elaborated by APCs or activated macrophages, in turn activates CD4+ lymphocytes and B cells.

cells to give off a series of humoral mediators. Other cytokines released by macrophages also may be important in graft destruction and include tumor necrosis factors beta (TNFβ; cachectin) and alpha (TNFα; lymphocytotoxin).[47] In addition TNF released locally by cells infiltrating the graft may (with IFNγ) stimulate further MHC antigen expression by the graft.

Activated CD4+ T cells are critical in the early phases of the rejection cascade because of their ability to elaborate several lymphokines. Some researchers have thought that the CD4+ Th lymphocyte population can itself be divided into at least two subclasses (Th$_1$ and Th$_2$), each of which elaborate distinct lymphokines with their own properties and actions (Fig. 3).[48] The most important, Th1-derived IL-2, stimulates activated T and B lymphocytes to differentiate and proliferate. Other interleukins (there are now eight recognized) stimulate B-cell maturation or effect cell populations in the bone marrow. Th1-derived IFNγ has several putative roles in immunoresponsiveness.[49] It amplifies the entire process by inducing and intensifying class I and class II MHC antigen expression on the graft, stimulates B cells to increase antibody production, and augments alloaggressiveness of previously uncommitted macrophages and monocytes. In addition it may increase adhesiveness of lymphocytes to an antigenic site by enhancing expression of LFA-1 on their surfaces.[40] In some systems IFNγ antagonizes activity of IL-1 and IL-4; however transfer of cloned material to an unresponsive host may produce acute rejection of otherwise well-functioning organ grafts.[50]

Early stimulation of T lymphocytes by antigen causes them to develop receptors on their surfaces for transferrin, insulin, IL-1, IL-2, and presumably other products as well.[51] The development of high-affinity surface receptors for IL-2 on most activated CD4+ and CD8+ T lymphocytes, some B cells, DCs, and macrophages has been thought particularly important in the rejection cascade. Binding of this lymphokine to its receptor is followed by internalization of the entire complex, which transduces the signal for proliferation and clonal expansion of the activated cell population and drives the entire rejection event forward. This antigen-activated cell population is relatively small; about 15% of infiltrating cells in rejecting rat cardiac grafts are IL-2R positive and more than 20%

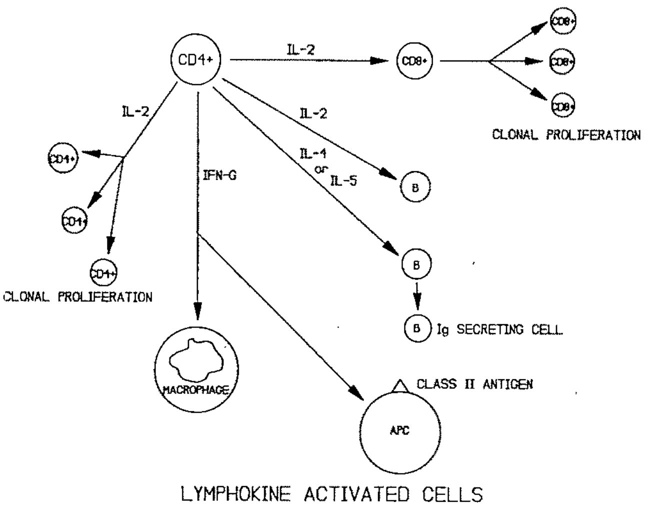


FIG. 2. Activated CD4+ lymphocytes produce several lymphokines, including gamma interferon (IFN-G), which stimulate other cell populations.

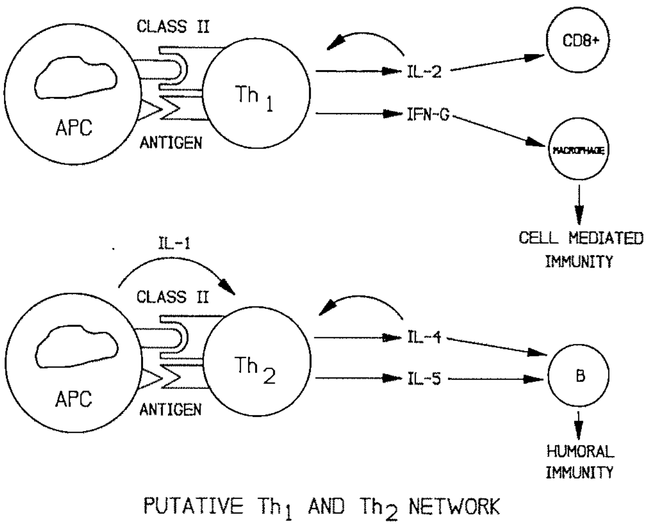


FIG. 3. Two putative Th subpopulations are thought to elaborate lymphokines differentially.

of lymphocytes express this receptor in a popliteal lymph node model.[13,52]

The importance of activated IL-2R–positive cells in rejection has been shown in several species, including humans, by specific anti-IL-2R MAbs.[53] Using these antibodies it has become clear that IL-2R are heterogeneous in terms of binding and structural characteristics; low, intermediate, and high affinity forms have been described. Interleukin-2R–targeted therapy prolongs skin and organ graft survival dramatically in some mouse strains.[54] In an extensive series of experiments in rat allograft models, the role of epitope defined on target cells was found to be critical; that is some antibodies directed against particular epitopes of the receptor complex prevent or reverse rejection; others directed against functionally different epitopes, although active *in vitro*, are therapeutically ineffectual. Monoclonal antibodies against different IL-2R epitopes also were found to be additive or even synergistic, suggesting more complete coverage of the three-dimensional molecular receptor structure.[53] In addition the effectiveness of antibody isotype may vary. At least in rats, the alloaggressiveness of IL-2R–positive cells is decreased by actual lysis, not merely by covering the receptor molecule.[55] As a result IL-2R–positive cells from the graft infiltrate were virtually eliminated by these antibodies, with significant increase in graft survival. In contrast cells with suppressor activity (Ts) were spared. Some anti–IL-2R MAbs act synergistically with subclinical doses of cyclosporine, an observation of potential clinical importance.[56] The potency of these MAbs in increasing graft survival in subhuman primates and in humans, although less dramatic than in smaller laboratory animals, also have been interesting enough to stimulate clinical trials.[57] At the very least, early use of such antibodies in human renal transplant recipients delay the first rejection event.

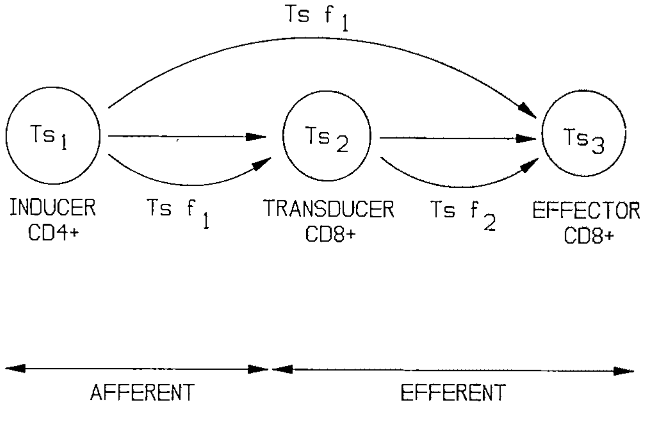
Regulation of Immune Responsiveness

After the rejection episode is completed and the graft has been destroyed, the host responses must return to baseline; thus intrinsic control mechanisms must arise to reverse the immune processes. As graft antigen expression progressively diminishes, clonal expansion of lymphocyte subpopulations slows with eventual reversion of the cells to their resting states; lymphokine transcription, messenger RNA-encoding IL-2R, and lymphokine production gradually cease. In addition suppressor mechanisms may be brought into play to reverse the inflammatory process.

The concept of suppression as an active immunoregulatory mechanism is intrinsic to the study of transplantation biology. It originated in studies of tolerance whereby fetal or neonatal animals, initially exposed to specific antigens, became permanently nonreactive to the same antigens when they were presented later in life.[58] Tolerant states then were found to be thymus dependent; specific immunologic unresponsiveness could be induced in the normal host by transfer of thymocytes from tolerant animals, while removal of such tolerance-inducing cells in bulk, as by thymectomy, may cause abrupt allograft rejection in several experimental models.[59,60] It has shown subsequently that various immunosuppressive modalities, both chemical and biologic, can inhibit the effector responses of graft recipients but spare cell populations with suppressor capabilities that contribute to the development of specific host unresponsiveness.[61]

T lymphocytes with suppressor characteristics presumably are critical in producing and maintaining immunologic homeostasis in a host that is exposed throughout its life to multiple antigenic stimuli. Although there is ongoing debate about their existence by basic immunologists, Ts (or at least cells with suppressor function) have been identified both *in vitro* and *in vivo* in various immunologic models and disease states. In transplantation models in which allografts survive for prolonged periods in unresponsive recipients, Ts often are demonstrable in the maintenance phase of unresponsiveness; indeed a common denominator of long-term host unresponsiveness seems to be the activity of such cells or their products. These experimental systems include neonatally induced tolerance, allograft recipients undergoing total lymphoid x radiation, or enhanced animals pretreated with antigen and/or antibody.[62] They also include engrafted animals conditioned with donor blood and anti-lymphocyte serum and those immunosuppressed with cyclosporine.[56,61]

It is interesting that in several models the CD4+ and CD8+ phenotypes shift between the early and later phases of unresponsiveness.[63] Because of our inability to differentiate between effector and suppressor cells by phenotype, it is possible that suppressor function can supersede effector activity in cells of the same phenotype; indeed this same cell may be responsible for both processes, depending on the circumstance of host immune stimulation. Thus Tc/s may not be only responsible for completing graft destruction but for halting the systemic effects of immunity. Alternatively more than one population of Ts may be responsible for inhibition of alloresponsiveness; indeed a cascade of suppression may exist that involves cell-to-cell contact and sequential activity of lymphocyte products, all orchestrated by cells differing in phenotype, MHC restriction, and allospecificity (Fig. 4).[64] Originally identified in a murine DTH model, the suppressor cell pathway has been shown to exist in transplant models by the demonstration of distinct T-cell subsets appearing during the course of prolonged rat renal allograft survival.[62] Three Ts subpopulations have been described: Ts_1 inducer/suppressor cells, Ts_2 transducer cells, and Ts_3



PUTATIVE SUPPRESSOR T CELL NETWORK

FIG. 4. The postulated suppressor cell network is shown.[64]

auxiliary or effector suppressor cells. Ts_1 and Ts_3 may bear antigen-binding receptors with idiotype-related determinants, whereas Ts_2 may have receptors for anti-idiotypes. Finally some Ts subsets may release factors that mediate both specific and nonspecific suppression by other cell subpopulations.

Thus the concept of a series of controlling or immunoregulatory mechanisms appearing as an integral part of the immune system is becoming increasingly appreciated. A balance must be maintained between effector mechanisms and suppression in acute host alloresponsiveness whereby allograft destruction ultimately is stopped by suppressor mechanisms and the animal restored to immunologic normalcy. The appearance of Ts in the later stages of rejection, regardless of phenotype, apparently represents such a host protective mechanism. Whether Ts that emerge during the rejection process represent the same populations as those governing graft acceptance in immunomodulated hosts is unknown, but seems likely. Nor is it known whether such cells are involved in the immune regulation that occurs throughout the life of an animal barraged constantly by environmental antigenic stimuli presented through skin or gut.

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The Influence of Intraoperative Hypotension and Perioperative Blood Transfusion on Disease-free Survival in Patients with Complete Resection of Colorectal Liver Metastases

RIAD N. YOUNES, M.D.,* ANDRE ROGATKO, PH.D.,† and MURRAY F. BRENNAN, M.D.*

From the Departments of Surgery and Biostatistics,†*
Memorial Sloan-Kettering Cancer Center,
New York, New York

An increased interest in surgical treatment of liver metastases from colorectal origin has evolved recently. However not all patients benefit from this approach, with early recurrence and death still being encountered. To evaluate clinical as well as perioperative factors that might significantly affect the outcome of patients with completely resected colorectal liver metastases, we examined 116 patients who underwent resection between September 1987 and August 1989. Median follow-up time was 13.2 months (0.6 to 31.4 months). The overall survival rate was 91% at 1 year and 75% at 2 years. Median survival was not reached. Median disease-free survival time was 11.5 months, with 49.4% and 21.2% of the patients being free of disease at 1 and 2 years, respectively. By univariate analysis, site of primary colorectal cancer, preoperative carcinoembryonic antigen (CEA) level, size of metastases, number of metastases, length of operation time, percentage mean arterial pressure, number of hypotensive episodes, duration of hypotensive episodes, and whole blood transfusion significantly affected recurrence rate following resection. However only site of primary tumor, CEA, number of metastases, and number of hypotensive episodes remained significant in the multivariate analysis. The most significant single factor that affected recurrence rate was the number of hypotensive episodes during the operative procedure. It is concluded that hypotensive episodes, even when well controlled, should be avoided during operation to maximize the chances of cure and prolong disease-free survival of patients with colorectal liver metastases.

SURGICAL RESECTION OF metastatic liver disease has recently become widely accepted, with reports of low operative morbidity and mortality rates for this procedure, along with its efficacy in affecting long-term survival of the patients.[1] However it has become clear that the improvement in survival is not wide spread, making it fundamental to identify the patients most likely to benefit from resection. Many reports that have discussed the influence of clinical prognosticators on the outcome of patients with liver metastases of colorectal cancer are controversial, mainly because of the small number of patients, the long interval between the time the first and last patients were entered, and the heterogeneity of the patients and their management.

In the present study we report a single institution experience with patients undergoing complete resection of colorectal liver metastases during a short period, September 1, 1987 to August 31, 1989. We evaluate preoperative clinical prognostic determinants as well as perioperative factors that could affect disease recurrence and survival.

Materials and Methods

During the period from September 1987 to August 1989, 133 patients underwent complete hepatic resection for colorectal metastases. Complete information in the medical records was not available for 17 patients and thus they were excluded from the analysis. We examined 116 patients for whom comprehensive data were available. Nine patients did not have complete information on all the variables analyzed (Table 1). All patients underwent resection after a preoperative workup and intraoperative surgical confirmation of the absence of discontiguous extrahepatic tumor. After the operation the patients were followed routinely. Median follow-up time was 13.2 months (range 0.6 to 31.4 months).

The following factors were considered for prognostic effect: age, site of primary colorectal cancer, Duke's stage of primary tumor, preoperative carcinoembryonic antigen

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Address reprint requests to Murray F. Brennan, M.D., Chairman, Department of Surgery, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021.

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TABLE 1. *Patient Characteristics and Distribution*

Variable	n	Mean	SD	Minimum	Maximum
Age (years)	116	59.3	11.5	29	87
CEA	109	97.1	341.5	0	3190
Number of metastases	116	1.8	1.4	1	8
Size of metastases (cm)	110	5.7	3.95	0.5	29
Interval from primary (months)	116	19.8	20.2	0	99.7
Operative time (min)	116	231.9	86.6	75	590
Baseline MAP (mmHg)	116	98.0	11.4	73.3	140
Minimum MAP (mmHg)	116	71.6	8.4	50	96.6
Per cent MAP	116	73.8	10.6	44.1	96
Duration hypotensive episodes (min)	116	30.1	28.4	0	120
Number hypotensive episodes	116	2.1	1.9	0	9
Blood loss (mL)	116	1964.0	1447.0	100	7000
Whole blood (U)	116	0.6	1.4	0	8
Packed cells (U)	116	2.9	4	0	26
Plasma (U)	116	5.9	7.5	0	48
Site of primary					
Rectum	30				
Sigmoid	29				
Left	24				
Right	24				
Stage of primary					
A	1				
B	32				
C	83				
Operation type					
Wedge	24				
Segmentectomy	28				
Left lobectomy	10				
Right lobectomy	47				
Trisegmentectomy	7				
Distribution of metastases					
Unilobar	99				
Multilobar	17				

CEA, carcinoembryonic antigen; MAP, mean arterial blood pressure; SD, standard deviation; U, units.

(CEA) levels, size of metastases (sum of the greater diameter of each individual nodule), number of metastases, distribution of nodules (uni- or multilobar), disease-free interval (time from primary to resection of hepatic metastases), type of hepatic resection (wedge, segmentectomy, left lobectomy, right lobectomy, trisegmentectomy), operative time, intraoperative blood loss, blood transfusion (whole blood, packed red blood cells, fresh frozen plasma) during the perioperative period (the day of surgery and the 72 following hours), and intraoperative hypotension.

Intraoperative hypotension was evaluated by determining the mean arterial pressure (MAP) from the anesthesia chart. The MAP was calculated by the following equation: (systolic pressure + 2x diastolic pressure)/3. Baseline MAP was defined as the pressure when the surgeon started the operation. Minimum MAP is the lowest MAP reached during the operation. This value (minimum MAP) is then expressed as a percentage of the baseline (percentage MAP). We defined hypotension in this study when MAP decreased to levels lower than 80% of baseline MAP. Subsequently we determined the number of hypotensive episodes (less than 80% baseline MAP) during the operation, as well as the total duration (in minutes) of the hypotensive episodes. From these determinations we evaluated the following parameters as prognostic factors: baseline MAP, minimum MAP, percentage MAP, number of hypotensive episodes, and overall duration of hypotensive episodes.

Statistical Analysis

To assess the dependence of death or recurrence on the variables analyzed, overall and disease-free survival rates were estimated for each variable by the Kaplan and Meier product-limit method.[2] Log rank test[3] was used to check dependence of overall and disease-free survival rates on each variable. Proportional hazard regression[4] was used to incorporate all of the explanatory variables in the same model. Forward stepwise procedure and likelihood ratio tests were used to select the variables with the greatest prognostic value. Cumulative hazard plots were generated to check visually the assumption of the proportionality of the hazard rates. Statistical analysis was performed using the BMDP computer software package.[5] The critical significance level of A = 0.05 was chosen.

Results

The distribution of patients by each variable analyzed is displayed in Table 1. Median blood loss was less than two units (Fig. 1), with approximately 25% of patients requiring no blood transfusion. Most patients did not receive whole blood (Fig. 2) or packed red blood cell transfusion (Fig. 3), but a few patients had major blood and blood product restoration (Table 1).

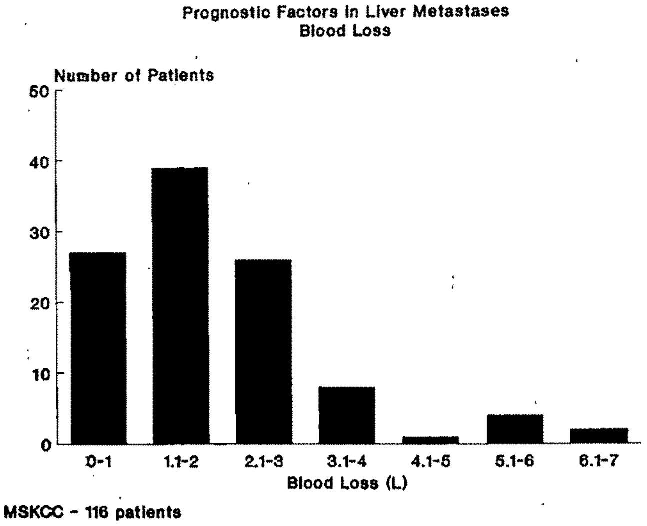

FIG. 1. Blood loss in patients undergoing complete hepatic resection for colorectal metastases. Median blood loss was less than two units.

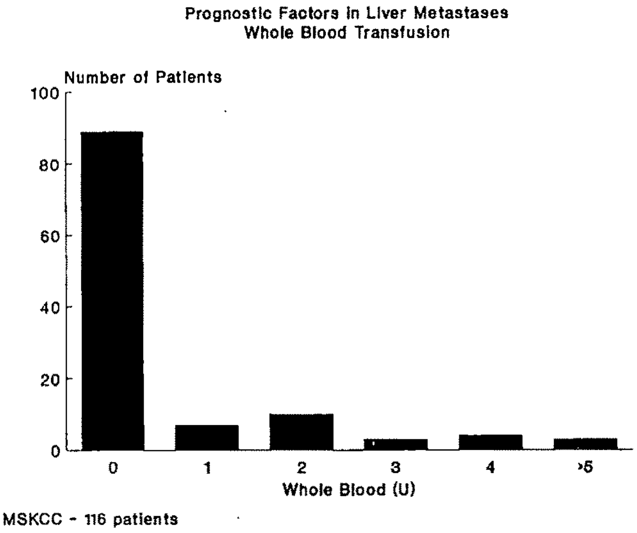

FIG. 2. Approximately 25% of patients required blood transfusion.

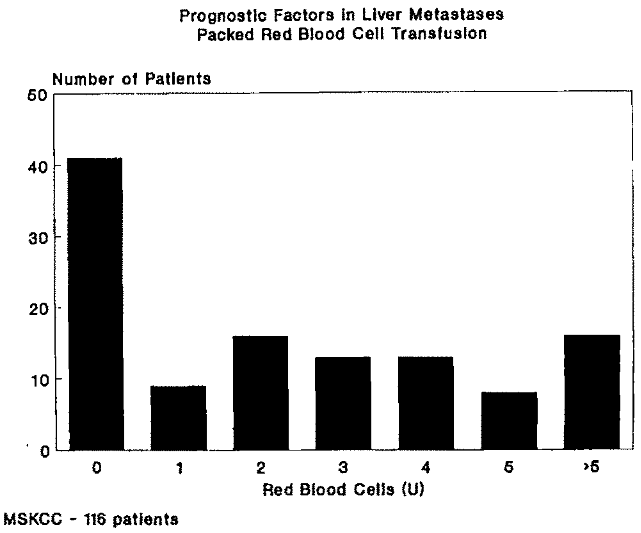

FIG. 3. Most patients did not receive packed red blood cell transfusion.

The median time to second recurrence after resection was 9.03 months (range, 1.1 to 27.4 months). Median disease-free survival time was 11.5 ± 2.5 months. Overall disease-free survival rate of the patients was 49.4% ± 5% at 1 year and 21.2% ± 5% at 2 years. The median survival after resection was not reached, with a 1-year survival rate of 91.3% ± 3.4%, and a 2-year survival rate of 74.7% ± 6.8%.

Twelve patients of 116 died during follow-up; consequently none of the variables significantly affected overall survival rate. The significance of each variable on disease-free interval (second recurrence) was analyzed in more detail and the results are displayed in Tables 2 and 3. By univariate analysis, site of primary colorectal cancer, preoperative CEA level (Fig. 4), size of metastases, number of metastases, length of operation time, percentage MAP, number of hypotensive episodes (Fig. 5), duration of hypotensive episodes (Fig. 6), and whole blood transfusion significantly affected recurrence rate after resection. By multivariate analysis, only site of primary, CEA, number of metastases, and number of hypotensive episodes remained significant (Table 2). The most significant single factor that affected recurrence rate was the number of hypotensive episodes during the operation (Fig. 5).

An equation derived from the analysis was determined to predict the relative risk of recurrence after hepatic resection of metastases:

Risk of Recurrence

$$= e\,[(\#\text{ of episodes} * 0.31) + (\#\text{ of metastases} * 0.21) + (\text{CEA} * 0.001) - (\text{Sigmoid/left} * 0.76)]$$

where: e = base of natural logarithm

CEA = Carcinoembryonic antigen level in ng/mL

Sigmoid/left: If primary is in sigmoid or left colon, enter 1.

If primary is in other site, enter 0.

The patients were equally distributed into four groups (each group containing approximately 25% of the patients) according to the calculated risk of recurrence: A < 1.23; B 1.23–1.81; C > 1.81–< 3.16; D ≥ 3.16. There was a significant difference in the recurrence rate among the four groups (Fig. 7).

Discussion

Colorectal carcinoma frequently metastasizes to the liver. Up to 35% of patients have apparent or occult me-

TABLE 2. *Significance of Evaluated Variables on Disease-free Survival (Critical Significance Level of A = 0.05)*

	Probability Value			
Variable	Univariate Analysis	Multivariate Analysis	Coefficient	SE
Age (years)	0.7827			
Site of primary	0.0269	0.0033	−0.7579	0.2596
Stage of primary	0.1393			
Disease-free interval	0.7320			
Carcinoembryonic antigen	0.0256	0.0232	0.0009	0.0003
Size of metastases (cm)	0.0467			
Number of metastases	0.0056	0.0213	0.2086	0.0835
Distribution	0.1800			
Type of operation	0.8793			
Operation time (min)	0.0198			
Blood loss (mL)	0.7436			
Mean arterial pressure (mmHg)				
Baseline	0.1773			
Minimum	0.0808			
Percentage MAP	0.0084			
Number of episodes	0.0001	<0.00001	0.3097	0.0708
Duration (min)	0.0006			
Whole blood (U)	0.0462			
Packed cells (U)	0.4809			
Plasma (U)	0.2337			

SE, standard error; MAP, mean arterial blood pressure; U, units.

TABLE 3. *Pearson Correlation Coefficients Among Variables–Coefficient Value (Probability Value)*

Variable	Site	CEA	No. Metastases	No. Episodes
Age (years)	0.1086 (0.2456)	0.2022 (0.0349)	−0.1048 (0.2758)	−0.0114 (0.9030)
Stage of primary	−0.2182 (0.0220)	0.1172 (0.2248)	0.1126 (0.2413)	0.0967 (0.3147)
Size of metastases	0.0387 (0.6875)	0.2049 (0.0326)	0.4520 (0.0001)	0.1415 (0.1402)
Distribution of metastases	0.0407 (0.6727)	−0.0817 (0.3980)	0.4268 (0.0001)	0.1205 (0.2097)
Interval from primary	0.2075 (0.0304)	−0.0616 (0.5265)	−0.1345 (0.1631)	−0.0175 (0.8563)
Type of operation	0.0911 (0.3307)	0.1213 (0.2088)	0.2024 (0.0339)	0.1270 (0.1740)
Time of operation	−0.0024 (0.9792)	−0.0416 (0.6672)	0.1521 (0.1126)	0.3906 (0.0001)
Baseline MAP (mmHg)	0.2648 (0.0041)	0.0570 (0.5560)	0.0725 (0.4513)	0.5145 (0.0001)
Minimum MAP (mmHg)	−0.0471 (0.6154)	0.1126 (0.2436)	0.0801 (0.4051)	−0.4582 (0.0001)
Percentage MAP	−0.2533 (0.0060)	0.0336 (0.7287)	−0.0104 (0.9136)	−0.7743 (0.0001)
Duration of hypotensive episodes (min)	0.1471 (0.1558)	−0.0490 (0.6125)	0.0820 (0.3940)	0.8866 (0.0001)
Blood loss (mL)	0.1883 (0.0429)	−0.0781 (0.4193)	0.1182 (0.2185)	0.2843 (0.0020)
Whole blood (U)	0.0447 (0.6331)	−0.0122 (0.8993)	0.0216 (0.8222)	0.1504 (0.1069)
Packed cells (U)	0.0921 (0.3254)	−0.0520 (0.5910)	0.1349 (0.1598)	0.2028 (0.0289)
Plasma (U)	0.0784 (0.4020)	−0.0776 (0.4220)	0.1535 (0.1094)	0.1635 (0.0795)

CEA, carcinoembryonic antigen; MAP, mean arterial blood pressure; U, units.

tastases at the time of laparotomy for the resection of a primary colon cancer.[6] Metachronous tumors occur within 2 years of resection of the primary disease in approximately 50% of all patients who develop hepatic metastases,[7] while more than 65% of the patients will have liver involvement by the time they die. Untreated patients have a poor prognosis, with median survival time between 4.5 and 15 months, with rare exceptions surviving more than 3 years.[8]

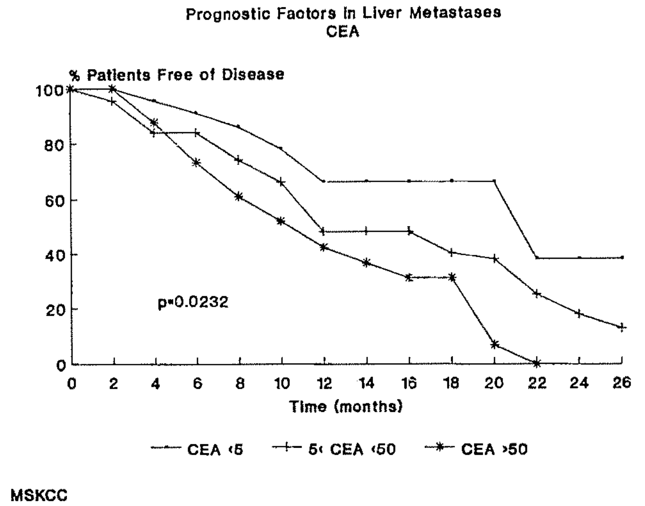

FIG. 4. Preoperative carcinoembryonic antigen levels.

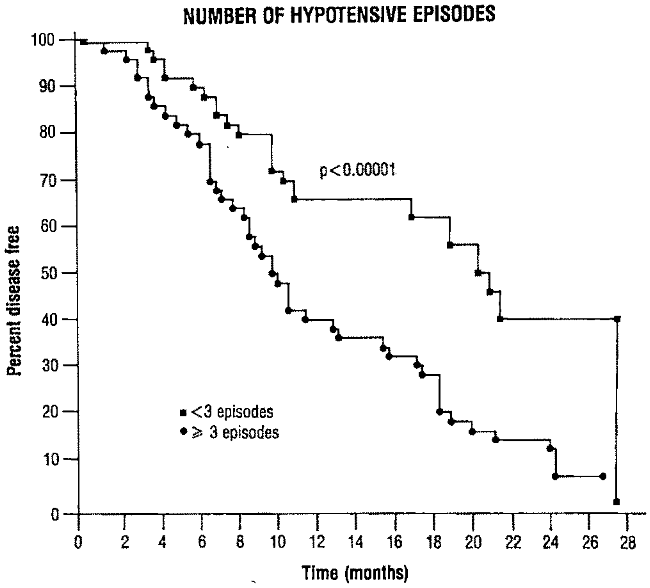

FIG. 5. The most significant single factor that affected recurrence rate was the number of hypotensive episodes ($p < 0.00001$).

Growing evidence suggests that surgical resection is currently the only effective treatment for selected patients with liver metastases of colorectal origin. However it is clear that not all patients benefit from resections because early postoperative recurrence is observed in many patients.[3] Detection of clinical factors that could predict the outcome after operation would improve the selection of patients.

The present study was undertaken primarily to investigate factors in the perioperative management of selected patients that could have prognostic influence. The predictive value of treatment variables was compared with that of clinical factors to identify the optimal treatment strategy for patients with colorectal liver metastases. Due to the relatively short follow-up and to the low mortality rate in our series, only disease-free survival (second recurrence) is discussed in depth in the present study. Overall survival in our patients was 91.3% and 74.7% at 1 and 2 years, respectively, after resection. These results compare favorably with previously reported series[1] but emphasize the considerable disparity between disease-free and overall survival.

To evaluate patient outcome, several variables were analyzed. Age was not a significant factor. Site of primary was shown to be a significant prognosticator in our series, with primary tumor originating in the left or sigmoid colon being associated with lower recurrence rates than primary

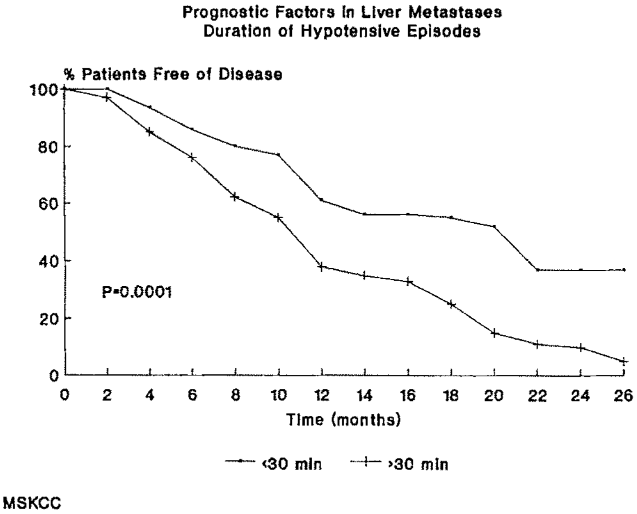

FIG. 6. Duration of hypotensive episodes (p = 0.0001).

tumor in other locations. These findings are in accordance with other studies,[9,10] but most series do not show any significant effect of site on outcome.[1,11-13]

In our study the risk of recurrence was not significantly affected by the stage of the primary colorectal cancer. Authors have reported conflicting results regarding the significance of Duke's classification on the outcome of patients with resected colorectal cancer metastatic to the liver, with some reports showing significant effect[1,9,12] while others show no effect.[10,13-15]

In our experience a short disease-free interval between the primary and liver recurrence did not significantly increase the likelihood of recurrence. Similar results have been shown in other reports.[1,12,16] However some investigators have suggested that patients with longer disease-free intervals between the primary occurrence and metastasis did better than patients with a shorter disease-free interval.[9,11] By univariate analysis the number of metastases and the size of the metastases (both used to quantify tumor burden) were shown to be significant predictors of recurrence in our study, but they were found to be interdependent. The coefficient of correlation between number of metastases and size of metastases was high (coefficient = 0.452, p = 0.0001). By multivariate analysis, however, only the number of metastases was selected as a significant determinant of outcome. This is an indication that tumor burden is an important factor. A higher number of metastases implies more diffuse spread of the disease, which theoretically increases the likelihood of other microscopic metastases being present in other sites, intra- or extrahepatic, with delayed growth and recurrence.[8,11] In a study by Nordlinger et al.,[17] the number of liver metastases resected did not affect postoperative outcome and survival for patients with less than four liver metastases resected was not different from survival in those with more than four resected.

Carcinoembryonic antigen is a valuable laboratory test for detecting hepatic metastatic disease in patients with colorectal cancer.[18] Preoperative CEA levels significantly affected the outcome of patients in our study. It was analyzed as a continuous variable and progressively higher levels were associated with increased incidence of recurrence. Other studies, however, do not show any significance of CEA as a prognostic determinant.[1,13]

As other studies have found, the type of resection *per se* did not influence survival in our patients. The extent of resection is dictated by the number, distribution, and size of metastases. Flanagan and Foster[16] noted that overall survival rate of 39% at 5 years following lesser operations of wedge resection and 'partial lobectomies' compared quite favorably with more extensive resections. Other authors[9,13,15] also have found that the simpler procedures offered better median survival time.

Recurrence was not affected significantly by the distribution of liver metastases in our study. Patients with uni- or multilobar involvement did not have a significantly different risk of recurrence after resection. When patients were stratified for tumor burden (number and size of metastases), the effect on recurrence and survival was independent of the distribution of the hepatic nodules, as long as the operative procedure encompassed all the disease with clear margins. Some authors, however, advocate that only those patients with solitary or unilobar metastases should be considered to be candidates for liver resection.[11,19]

To evaluate the second aspect of our study, the prognostic influence of patient management factors, we concentrated on factors that affect the response of the patient to microscopic neoplasia present after completion of liver

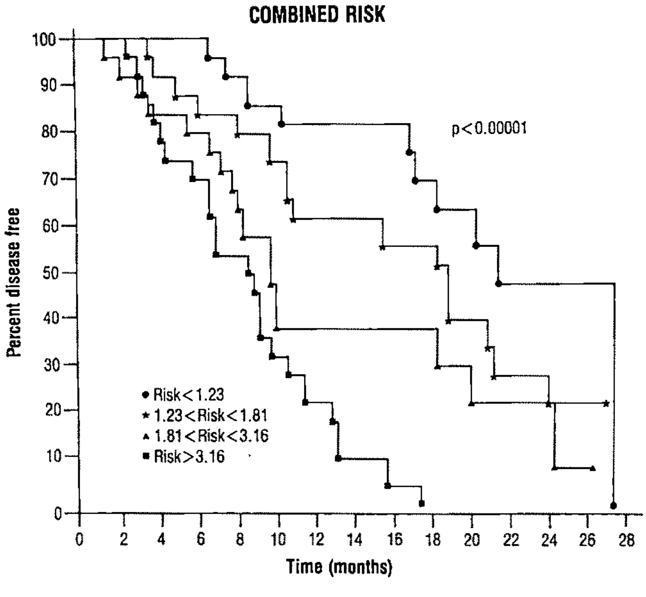

FIG. 7. Disease-free survival in patients separated into combined relative risk groups. Risk was calculated using the equation described in Results.

resection. It is known that immunocompetence of the patient is important in the development and rate of growth of tumors. Two factors that occur during management of patients with liver metastases are classically shown to be modulators of immunity: transfusion of blood-derived products and hypovolemia.

The administration of blood reduces the incidence and severity of graft rejection in kidney transplant recipients.[20] Transfusions exert an immunosuppressive effect on the host through yet unknown mechanisms. Blood transfusion in animal studies diminishes cell-mediated immunity, decreases macrophage migration, and increases the production of immunosuppressive prostaglandin E.[21] Recently blood transfusions were shown to have detrimental effects on patients harboring malignant tumors. Reports have shown that in patients with various malignancies,[22-24] there was a higher rate of tumor recurrence in those that received transfusion. However these findings are not uniformly observed.[25] A previous study by Stephenson et al.[26] showed a significant influence of blood transfusion on the outcome of 59 patients with colorectal liver metastases operated on during a 10-year period. Our study, like theirs, showed that only transfusion of whole blood significantly affected recurrence. This effect, however, was seen only in the univariate analysis. By multivariate analysis, none of the blood products transfused affected disease-free survival. There was no multivariate analysis in the study of Stephenson et al.[26]

One other factor analyzed in our study surprisingly has been completely overlooked in the majority, if not all, the studies that evaluate the effect of transfusion on tumor growth and recurrence. This was the occurrence of intraoperative hypotension. We observed that the number of hypotensive episodes was the single most significant prognosticator. The duration of hypotension also was a significant factor by univariate analysis, but it was highly correlated with the number of hypotensive episodes (correlation coefficient = 0.886, $p = 0.0001$). Consequently multivariate analysis selected only the number of episodes, rather than the duration of hypotension, as the significant factor.

Blood loss and hypovolemic shock have been shown to affect the immune system significantly.[27,28] These events depress mitogen-induced lymphocyte proliferation[29] as well as the production of interleukin-2.[30] In an experimental study performed in our laboratory, hypovolemic events significantly enhanced tumor growth independent of blood transfusion.[31] These results emphasize that careful hemodynamic management of the patient to avoid hypovolemic episodes during treatment is fundamental. Following careful selection of the patients with the highest potential of curability, the surgeon must avoid detrimental variables that are preventable.

Our results demonstrate that some variables (number of metastases, CEA level, site of primary tumor) can help the surgeon to identify patients with a greater likelihood of recurrence and other treatment variables (*e.g.*, number of hypotensive episodes) that could further affect recurrence rate. Prospective clinical studies, although difficult to conduct, should be designed to define management optimization, perhaps including patients with clinically unfavorable factors in preoperative neoadjuvant treatment, and patients with unfavorable treatment variables (*e.g.*, hypotension) in postoperative adjuvant treatment.

Acknowledgments

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Intrahepatic Recurrence After Resection of Hepatocellular Carcinoma Complicating Cirrhosis

JACQUES BELGHITI,[*] YVES PANIS,[*] OLIVIER FARGES,[*] JEAN PIERRE BENHAMOU,[†] and FRANCOIS FEKETE[*]

From the Service de Chirurgie Digestive,[*] Service d'Hépatologie,[†] Hôpital Beaujon, Clichy, Université Paris VII, Paris, France

To determine whether a careful evaluation of tumor extension by preoperative computed tomography scan after intra-arterial injection of ultrafluid lipiodol and by intraoperative ultrasound examination reduced the recurrence rate of hepatocellular carcinoma after resection, a series of 47 cirrhotic patients with a single tumor operated on from 1984 was studied. Alphafetoprotein level was less than 100 ng/mL in 26 patients (55%), size of the tumor was less than 5 cm in 28 patients (59%), and capsule was present in 30 patients (63%). The resection was performed with free margin measuring 1 cm or more. The overall cumulative survival rates at 3 and 5 years were 35% and 17%, respectively. Intrahepatic recurrence was observed in 28 patients (60%), located less than 2 cm from the resection margin in only four patients. The cumulative intrahepatic recurrence rate at 3 years was 81% and was significantly higher in patients with tumor ≥ 5 cm and in patients with preoperative alphafetoprotein level of ≥100 ng/mL. In this series the cumulative intrahepatic recurrence rate at 5 years was 100%. This high recurrence rate after resection, even with careful evaluation of tumor extension, indicates that liver transplantation might be envisaged for the treatment of cirrhotic patients with resectable hepatocellular carcinoma.

THE LONG-TERM results of resection of hepatocellular carcinoma (HCC) in cirrhotic patients have been disappointing, primarily because of the high rate of postoperative intrahepatic recurrence.[1,2] This might be due, at least in part, to undetected and therefore unremoved tumorous tissue at surgery.[3] Some imaging procedures, including preoperative computed tomograph (CT) scan after intra-arterial injection of ultrafluid lipiodol and intraoperative ultrasound (US) examination have a high sensitivity for detecting tumorous tissue within the liver.[4] From 1984 we systematically used these imaging procedures in the management of HCC. The purpose of the present study was to determine whether the recurrence rate after resection might be reduced by these imaging procedures in cirrhotic patients with an asymptomatic single HCC.

Patients and Methods

Among 68 patients who underwent hepatic resection for HCC at Hôpital Beaujon from 1984 to 1989, 47 patients having the following characteristics were studied: (1) all the patients were adults; (2) all of them were suffering from cirrhosis (histologically proved); (3) all of them were suffering from HCC (histologically proved); (4) hepatocellular carcinoma was asymptomatic and was detected by US examination and/or by increase in serum alphafetoprotein level (more than 100 ng/mL); (5) the preoperative arteriogram and CT scan after intra-arterial injection of ultrafluid lipiodol showed a single tumor; (6) the tumor was resected with a margin of 1 cm or more; (7) no residual tumor was detected by intraoperative US examination in the remnant liver after resection; (8) the survival time after resection was more than 6 months.

Among the 47 patients meeting the above criteria, there were 38 men and 9 women; their ages ranged from 35 to 76 years. The cause of cirrhosis was chronic alcoholism in 18 patients, chronic infection with hepatitis B virus in 19, idiopathic hemochromatosis in 5, and indeterminate in 5. Alphafetoprotein level was less than 100 ng/mL in 26 patients and more than 100 ng/mL in 21. According to the classification of Child modified by Pugh et al.,[5] 31 patients (66%) belonged to class A, 15 (32%) to class B, and 1 (2%) to class C. The diameter of the tumor (measured in the resected liver) was less than 5 cm in 28 patients, 5 to 10 cm in 12 patients, and more than 10 cm in 7 patients. The tumor was not encapsulated in 17 patients and encapsulated in 30. Resection consisted of tu-

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Address reprint requests to Prof. J. Belghiti, Service de Chirurgie Digestive, Hôpital Beaujon, 92118 Clichy, France.

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morectomy in 7 patients, removal of 1 segment of the liver in 18, and removal of 2 segments or more in 22.

All the 47 patients were examined every 3 months in the first 2 postoperative years and then every 6 months. Examination included liver tests, measurement of alphafetoprotein level, chest x-ray, and liver US. When recurrence was suspected, the patient was readmitted to the hospital and hepatic arterial angiography was performed. Intrahepatic recurrence was classified as recurrence arising in the vicinity of the resected area or as recurrence developing 2 cm or more from the resection margin. All the patients were followed for 6 months or more. No patient had hepatic arterial embolization before resection; no chemotherapy was used after operation. When recurrence was recognized, the choice for the treatment was based on the location of the recurrent tumor within the liver, the clinical condition of the patients, and the results of liver tests. In the 30 patients with recurrence, reoperation was performed in 3 (including liver transplantation in 1); intra-arterial chemotherapy was administered in 7; and no treatment was given in the remaining 20 patients.

Survival rate and cumulative intrahepatic recurrence rate after resection were calculated using the Kaplan–Meier method. Survival curves were compared using the log rank test.

Results

Of the 47 patients, 17 were alive at the time of submission of this paper (August 1990). The cumulative survival rates were 62% at 1 year, 47% at 2 years, 35% at 3 years, 25% at 4 years, and 17% at 5 years. Of the 30 patients who died, tumor recurrence was the cause of death in 21. Nine patients died of liver failure without evidence of tumor recurrence from 6 to 15 months after resection; 8 belonged to class B or C and 1 to class A. Although 17 of the 47 patients were still alive, 9 patients living from 6 to 60 months after resection had a recurrence. The other eight patients were living from 6 to 40 months without apparent recurrence.

Intrahepatic or extrahepatic recurrence of the tumor was recognized in 30 patients (64%). In two patients only the lungs or bones were involved. Of these 30 patients, tumor recurrence was confined to the liver in 28: intrahepatic recurrence consisted in multiple nodules in 21 and a single nodule in 7. The recurrent tumor or tumors were located less than 2 cm from the resection margin in four patients and more than 2 cm from the resection margin in 24.

Intrahepatic tumor recurred within 6 months after surgery in 5 patients, 6 to 12 months in 11, 13 to 24 months in 6, and more than 2 years in 6 patients. The interval from the time of recognition of tumor recurrence and death is set out in Table 1: among the 30 patients with tumor recurrence, 17 survived more than 6 months, 7 more than 1 year, and 4 more than 2 years. The patients who were reoperated on for a second hepatic resection and the patients who were treated by chemotherapy had a longer survival time than those not reoperated and those not treated by chemotherapy. The outcome of the patients alive after surgery showed that 27, 20, 7, 3, and 1 patients were alive at 1, 2, 3, 4, and 5 years, respectively. However the intrahepatic recurrence rate was observed in 11 (40%), 11 (55%), 4 (57%), 2 (67%), and 1 (100%), respectively. As showed in Figure 1, the cumulative intrahepatic recurrence rate was 42% at 1 year, 61% at 2 years, 81% at 3 years, 87% at 4 years, and 100% at 5 years.

TABLE 1. *Survival According to the Treatment Used in Patients with Tumor Recurrence*

Treatment	Number of Patients Surviving After Recognition of Tumor Recurrence		
	6 Months	1 Year	2 Years
Resection (n = 3)*	3	2 }†	2
Chemotherapy (n = 7)‡	6	4 }	2
No treatment (n = 20)	8	1†	

* Including liver transplantation in one patient.

† Administration of chemotherapeutic agents suspended in ultrafluid lipiodol through feeder arteries.

‡ These two values are significantly different ($p = 0.02$).

The cumulative intrahepatic recurrence rate according to the size of the tumor, the presence or absence of a capsule, and preoperative alphafetoprotein level are shown in Figures 2 to 4. At 3 years the cumulative intrahepatic recurrence rate was significantly higher in patients with a tumor diameter of 5 cm or more (91%) than in those with a tumor diameter less than 5 cm (78%) ($p < 0.05$). At 3 years the cumulative intrahepatic recurrence rate in patients with nonencapsulated tumor (100%) was higher than in patients with encapsulated tumor (68%). These differences, however, were not statistically significant ($p < 0.1$). At 3 years cumulative intrahepatic recurrence rate was significantly higher in patients with a preoperative alphafetoprotein level of more than 100 ng/mL (100%) than in those with a preoperative level of alphafetoprotein of less than 100 ng/mL (69%) ($p < 0.01$). The serial changes in alphafetoprotein level after resection are shown in Table 2. Preoperative level of alphafetoprotein was high in 21 patients. After resection alphafetoprotein level decreased in all the patients. Of these 21 patients, 11 had an intrahepatic tumor recurrence and in parallel an increased alphafetoprotein level, and four had a recurrence without elevation of alphafetoprotein. Preoperative level of alphafetoprotein was normal in 26 patients. Of these 26 patients, nine had a tumor recurrence without elevation of alphafetoprotein and four had a tumor recurrence and in parallel an increased alphafetoprotein level.

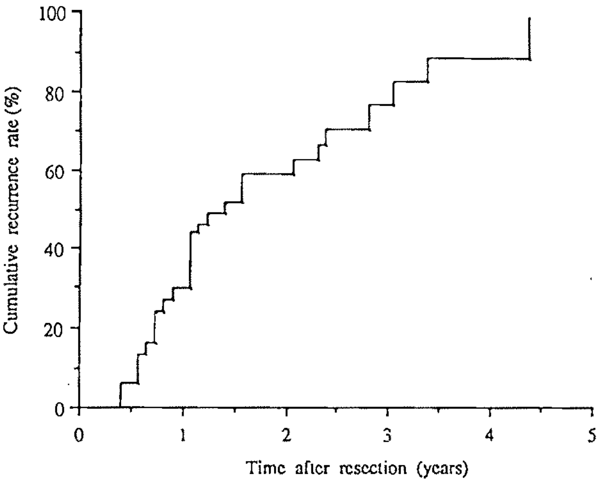

FIG. 1. Cumulative intrahepatic recurrence rate after resection of hepatocellular carcinoma in cirrhotic patients.

Discussion

Our data show that the cumulative intrahepatic recurrence rate reached 100% at 5 years after resection of a single HCC in cirrhotic patients. Tumor recurrence took place predominantly in the liver and was mainly located away from the resection margin.

The intrahepatic recurrence rate of 60% in this series was comparable to that reported in another series of patients after resection of HCC associated with cirrhosis.[3,6] In our patients surviving after resection, detection of intrahepatic recurrence by measurement of alphafetoprotein level and US at regular intervals showed that the cumulative recurrence rate increased progressively and reached 100% at 5 years. Long-term survival can be observed in a few patients with recurrence, particularly in those treated by a second hepatic resection or by intra-arterial chemotherapy.[7,8] Factors influencing the rate of recurrence were the size of the tumor, encapsulation of the tumor, and the preoperative level of alphafetoprotein. The preoperative high level of alphafetoprotein as a predictor of poor postoperative prognosis in this series has been previously reported.[2] After transplantation for HCC, a high preoperative level of alphafetoprotein was found to be an important factor influencing the rate of recurrence.[9] Our results indicate that the intrahepatic tumor recurrence rate in patients with nonencapsulated tumors was higher than in those with encapsuled tumors.[10,11]

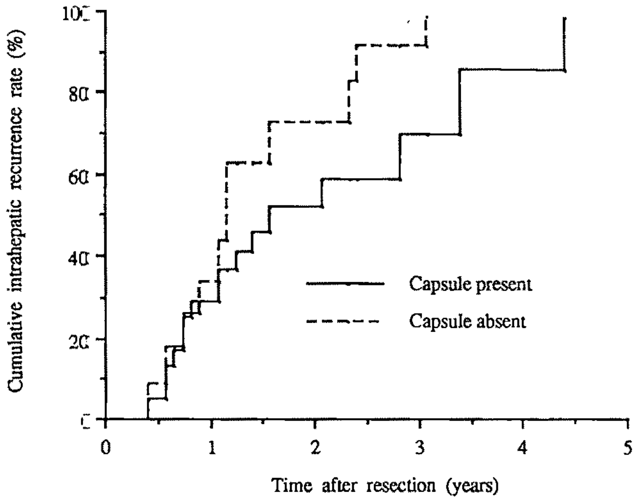

FIG. 3. Cumulative intrahepatic recurrence rate after resection of hepatocellular carcinoma according to the presence of a capsule.

Recurrence was located mostly in the remnant liver in this series, as well as in other series,[3,6] and might be due to (1) inadequate resection of the original tumor, (2) unrecognized multifocal HCC, and (3) multicentric origin of HCC in cirrhotic patients.

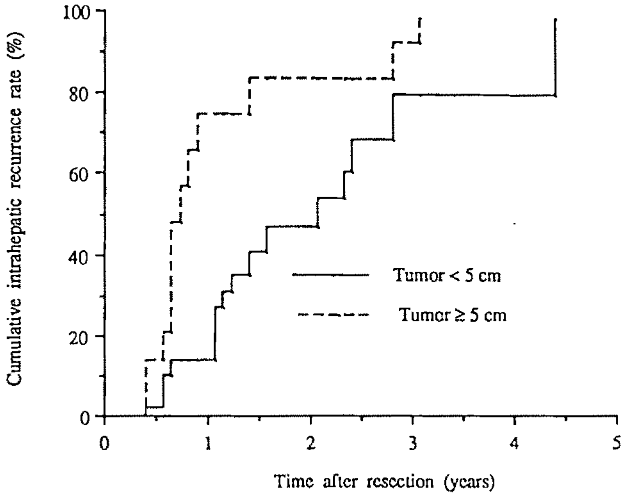

FIG. 2. Cumulative intrahepatic recurrence rate after resection of hepatocellular carcinoma according to the size of the tumor.

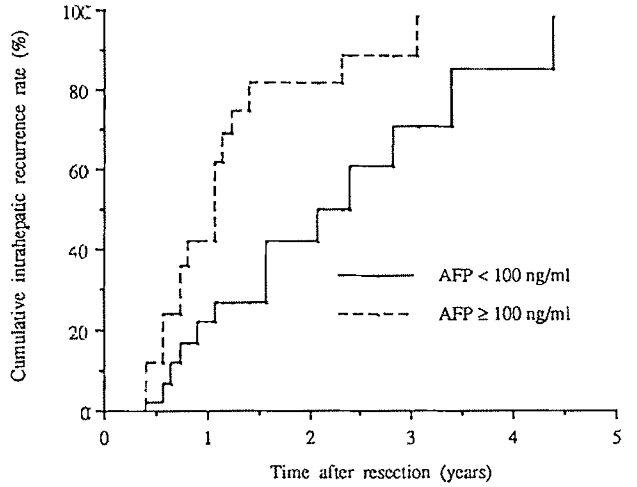

FIG. 4. Cumulative intrahepatic recurrence rate after resection of hepatocellular carcinoma according to the preoperative level of alphafetoprotein.

TABLE 2. *Serum Alphafetoprotein (AFP) Before Resection and After Recognition of Intrahepatic Tumor Recurrence*

AFP Before Resection (Number of Patients)	Patients with Intrahepatic Recurrence	Number of Patients with AFP Values After Detection of Recurrence	
		<100 ng/mL	≥100 ng/mL
<100 ng/mL (n = 26)	13	9	4
≥100 ng/mL (n = 21)	15	4	11

Recurrence might be due to inadequate resection of the original tumor.[6] The serial screening of cirrhosis has increased the presence of small neoplasic lesions; in those patients minor hepatic resection has been preferred to reduce the risk of postoperative liver failure.[12] The high cumulative intrahepatic recurrence rate observed could be the consequence of minor resection, especially in patients with large tumors.[3] In our series the cumulative intrahepatic recurrence rate was higher in patients with a tumor larger than 5 cm, as compared to those with tumors less than 5 cm. A free margin of parenchyma has been regarded as a prognostic factor in terms of tumor recurrence.[1] Resection with a 1-cm or greater free margin, as performed in this series, provided an effective treatment of HCC in cirrhotic patients for two reasons. First the 3-year survival rate of 35% observed in this study was similar to others.[2,13] Second, of 28 patients with intrahepatic recurrence of HCC, only 4 patients (14%) had a recurrence near the resected hepatic stump. The remaining 24 patients had either a nodular recurrence in the other segments away from the resected margin or a widespread multinodular recurrence in the liver remnant.

Tumor recurrence might be due to unrecognized multifocal hepatocellular carcinoma. In this series preoperative staging, including arteriography with ultrafluid lipiodol, CT, and intraoperative US, was performed in all patients. This procedure has reduced the number of patients with unrecognized multifocal HCC. Preoperative examination of the patients using arteriography with ultrafluid lipiodol injection and a CT scan 8 to 10 days latter provides the best investigation for detecting other tumors.[4] Intraoperative US provides the most accurate intraoperative exploration for detecting other intrahepatic tumors. Intraoperative US facilitated precise resection with a margin equal or superior to 1 cm. All of our patients included in this series had such preoperative and intraoperative investigation and support the curability of our resections.

Hepatocellular carcinoma may be of multicentric origin in cirrhotic patients.[14] Recurrence was detected more than 2 years after surgery in six patients. The long interval from resection to recurrence, the location of intrahepatic recurrence away from the surgical margin, and the difference in alphafetoprotein production after resection are consistent with the view that recurrence developing after resection might be, at least in some patients, new primary tumors in the liver remnant.

Although in selected patients with small encapsulated tumors and low alphafetoprotein level long-term survival can be expected after resection, liver transplantation should be envisaged in the treatment of cirrhotic patients with resectable tumor. In patients with poor liver function, transplantation obviously is the adequate treatment; among our 16 class B or C patients who died after discharge of the hospital, eight died of causes directly related to cirrhosis without tumor recurrence. Even in cirrhotic patients with good liver function, transplantation similarly might be envisaged because of the high rate of intrahepatic recurrence of HCC.

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Radiolabeled Antibody Imaging in the Management of Colorectal Cancer

Results of a Multicenter Clinical Study

RALPH J. DOERR, M.D.,* HANI ABDEL-NABI, M.D.,† DAVID KRAG, M.D.,‡ and EDITH MITCHELL, M.D.§

Presurgical colorectal cancer patients (n = 116) received single intravenous infusions of 1 mg of CYT-103 (OncoScint® CR103), an immunoconjugate of monoclonal antibody B72.3, radiolabeled with ^{111}In. Following gamma camera imaging, 103 patients underwent an operative procedure: 92 had primary or recurrent colorectal carcinoma, 1 patient evaluated for recurrence of colorectal cancer had a second primary malignancy (small cell lung), and 10 patients had no demonstrable evidence of malignancy. ^{111}In-CYT-103 immunoscintigraphic findings were consistent with the pathologic diagnoses for 70% of patients with colorectal cancer and 90% of disease-free patients. Antibody imaging contributed to surgical decision making through the detection of occult disease (12% of patients) and the confirmation of localized, potentially resectable disease without regional or metastatic spread. Seven patients (6%) experienced adverse effects, primarily fevers and itching, and 33% of patients developed antibodies to murine immunoglobulin after administration of ^{111}In-CYT-103. The results of this study suggest that ^{111}In-CYT-103 is a useful diagnostic tool for the presurgical evaluation of colorectal cancer patients.

THE PROGNOSIS OF patients with colorectal cancer is related to the stage of disease at the time of diagnosis.[1-3] Thus the diagnosis of primary disease by barium enema, sigmoidoscopy, or colonoscopy with biopsy should be followed by staging procedures to determine the extent of tumor.[4] Current systems for staging colorectal malignancies depend primarily on surgical and pathologic findings. However there are limitations in the surgeon's ability to detect disease during operation.[5] An accurate, noninvasive method of staging colorectal tumors would permit precise individual treatment to be planned before surgery.[2]

One potential application in the presurgical staging of colorectal cancer is the use of monoclonal antibodies directed against tumor-associated antigens and radiolabeled with gamma-emitting isotopes. Such agents have been administered to cancer patients to identify the anatomic distribution of a malignancy using immunoscintigraphy.[6,7]

Monoclonal antibody B72.3 has been investigated as a tumor imaging agent because of its pattern of extensive reactivity with mucin-producing adenocarcinomas and its limited reactivity with nonmalignant adult tissues.[8] Because TAG-72, the tumor-associated glycoprotein target of monoclonal antibody B72.3, reacts with up to 94% of colon adenocarcinomas, clinical studies of B72.3 immunoconjugates have been conducted in patients with colorectal cancer.[9-12] In one study in which ^{131}I-labeled B72.3 was administered intravenously to 20 patients with colorectal cancer, 70% of tumor lesions demonstrated radiolocalization that was at least three times that of the surrounding normal tissue.[9,10] In a second study conducted by the same investigators,[11] 8 of 12 patients had positive gamma camera scans after intraperitoneal administration of the radiolabeled monoclonal antibody; in seven of these patients, the images showed an excellent correlation with subsequent surgical findings. Importantly tumor lesions detected in 3 of the 12 patients in the latter study had been missed by conventional diagnostic tests. In a separate series, ^{131}I- or ^{111}In-labeled B72.3 was administered to 30 patients being evaluated for recurrence of colorectal carcinoma.[12] In this series immunoscintigraphy detected all five cases of local recurrence and three of five cases of liver metastases.

We present the results of a multicenter clinical trial in presurgical patients with primary and recurrent colorectal carcinoma using ^{111}In-CYT-103, an ^{111}In-labeled im-

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From the Department of Surgery,* Buffalo General Hospital, and the Nuclear Medicine Department,† Veterans Administration Medical Center, State University of New York at Buffalo, Buffalo, New York; the Division of Surgical Oncology,‡ University of California at Davis, Davis, California; and the Division of Hematology/Oncology,§ University of Missouri, Columbia, Missouri

Supported by a grant from Cytogen Corporation, Princeton, New Jersey.

Address reprint requests to Ralph J. Doerr, M.D., Department of Surgery, Buffalo General Hospital, 100 High St., Buffalo, NY 14203.

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munoconjugate of B72.3 prepared using a unique site-specific conjugation method.[13] We have evaluated the safety profile, imaging performance, and ability of this investigational diagnostic agent to contribute to the management of presurgical patients with colorectal cancer.

Materials and Methods

Purified, cell culture-produced monoclonal antibody B72.3 was obtained from Celltech Ltd. (Berkshire, United Kingdom) and site specifically conjugated with the linker-chelator complex, glycyl-tyrosyl-(N,ϵ-diethylenetriaminepentaacetic acid)-lysine (GYK-DTPA; JBL Scientific, Inc., San Luis Obispo, CA), at oxidized carbohydrate sites on the constant region.[13] The resulting immunoconjugate, B72.3-GYK-DTPA (OncoScint® CR103 or CYT-103; Cytogen Corp., Princeton, NJ), was concentrated by ultrafiltration and the monomeric antibody fraction was selected by gel filtration chromatography and filtered through a sterile 0.22-μm filter. Single doses of 1 mg of the CYT-103 solution were aseptically filled into individual vials and stored at 4°C.

Presurgical, adult ($\geq$21 years old) patients with suspected or biopsy-proved primary or recurrent colorectal carcinoma were eligible for participation in this multicenter study. All patients were required to provide written informed consent, and the study protocol was approved by the institutional review board of each participating study site. Pregnant women and patients who either had received antitumor therapy within the past 4 weeks, had a Karnofsky performance status of less than 60%, had previously received a murine antibody, or had a second primary malignancy were excluded from the study.

Patients who fulfilled the enrollment criteria noted above were administered single intravenous doses of ^{111}In-CYT-103. Doses of radiolabeled antibody conjugate were prepared by adding a buffered solution of approximately 5.5 mCi of $^{111}InCl_3$ (Amersham Corp., Arlington Heights, IL) to the vial containing the CYT-103 dose. The solution was mixed and incubated at room temperature for 30 minutes. The ^{111}In-CYT-103 was tested for free ^{111}In using an instant thin-layer chromatography procedure,[14] filtered through a 0.22-μm sterile filter, and infused over approximately 5 minutes.

Planar gamma camera images, including anterior and posterior projections of the pelvis, abdomen, and thorax, were obtained using large field-of-view cameras with parallel hole medium-energy collimators. Imaging was performed on at least two occasions between 2 and 7 days after ^{111}In-CYT-103 administration and separated by at least 24 hours. Single photon emission tomographic imaging was to be performed during one of the imaging sessions. The gamma camera scans were interpreted prospectively during the course of the clinical trials by the on-site nuclear medicine physicians. The results of immunoscintigraphy and the findings of other presurgical tests, including computed tomographic (CT) scans of the abdomen and pelvis (95 patients), chest x-ray (101 patients), barium enema (28 patients), colonoscopy (56 patients), magnetic resonance imaging (6 patients), and liver–spleen scan (12 patients), were recorded.

Surgery was to be performed after the completion of the two imaging sessions. The surgeon was apprised of the results of the presurgical workup, including the immunoscintigraphic findings. During surgery an attempt was made to confirm with tissue biopsy all potential lesions indicated by the scanning procedures. Suspected extra-abdominal disease sites were evaluated using biopsy procedures, as appropriate. Histopathologic examination and immunohistochemical analysis to quantitate the TAG-72 expression were performed on tissues obtained during surgery or biopsy.

Baseline serum levels of TAG-72 were determined using the CA 72-4 Radioimmunoassay (Centocor, Malvern, PA). Safety assessments included routine hematology, blood chemistry, and urinalysis determinations and vital sign measurements. Serum titers of human anti-mouse antibodies were measured using the ImmuSTRIP HAMA® Test System (Immunomedics, Warren, NJ), a direct enzyme-linked immunosorbent assay.[15]

Imaging performance parameters, including sensitivity, specificity, and accuracy, were tabulated on a per-patient and, when possible, per-lesion basis for antibody imaging and CT scanning. The study sample size was sufficient to estimate the sensitivities of immunoscintigraphy and CT imaging within ±10%. The per-lesion sensitivity of immunoscintigraphy and CT imaging for liver and extrahepatic lesions was compared using McNemar's test. Fisher's exact test and the extended Mantel-Haenszel procedure were used to determine the effect of various factors (*e.g.*, tumor location and size, tumor antigen expression, and so on) on imaging performance. Data are expressed as means ± standard deviation, and statistical significance is indicated by probability values of 0.05 or less.

Results

Imaging Performance

A total of 116 patients were enrolled in this trial at 24 study sites. The patient population was predominantly white (92%) and male (61%) and had a mean age of 64 years (range, 37 to 88 years). Sixty-two patients (53%) were evaluated for primary colorectal cancer and 54 (47%) were evaluated for recurrent disease. Each patient was administered a single intravenous infusion of 1 mg of CYT-103 radiolabeled with 4.21 ± 0.68 mCi of ^{111}In. After gamma camera imaging studies, 103 of the 116 patients underwent a biopsy or surgical procedure for diagnosis and/or treatment. Surgical and histopathologic findings revealed that 92 of these patients had colorectal adenocarcinoma, one patient evaluated for a recurrence of colorectal cancer had a second primary malignancy (small cell lung), and the remaining 10 patients were free of disease. The performance of immunoscintigraphy in these patients, based on prospective interpretations of the gamma camera scans by the nuclear medicine specialists at each study site, is summarized in Table 1.

Antibody imaging correctly identified at least 1 surgically confirmed tumor lesion in 64 of the 92 patients with adenocarcinoma (70% sensitivity), and antibody scans were negative for 9 of 10 patients who were free of malignancy (90% specificity). Therefore antibody imaging provided correct diagnoses for 73 of 102 patients, for an overall accuracy rate of 72%. In addition the ^{111}In-CYT-103 did not localize to the small cell lung tumor in the remaining patient, thus helping to rule out a recurrence of colorectal carcinoma in this patient. In the 92 patients with colorectal cancer, immunoscintigraphy detected 65% of the adenocarcinoma lesions. Five additional areas of abnormal antibody uptake consistent with tumor lesions were evaluated histopathologically and found to be nonmalignant tissues. Of these false-positive findings, three specimens were inflammatory lesions, one was a tubulovillous adenoma, and one was normal tissue. Immunohistochemical analysis, which was performed for three of the five tissues, revealed that one of the inflammatory lesions contained TAG-72 antigen, and the normal tissue and one other inflammatory lesion did not contain TAG-72.

^{111}In-CYT-103 immunoscintigraphy detected occult disease, *i.e.*, tumor lesions that were not detected by physical examination or by standard presurgical diagnostic tests conducted before antibody imaging, in 11 of the 92 patients (12%) with surgically confirmed adenocarcinoma. Six of these patients were being evaluated for suspicion of disease recurrence based on clinical findings or elevated carcinoembryonic antigen (CEA) levels. Occult lesions were detected in five patients with primary colorectal cancer. In three of these patients, the antibody scans detected more extensive regional or metastatic disease, including bony metastasis, carcinomatosis, and a mid-abdominal lesion. In the other two patients, antibody imaging correctly located primary tumor lesions that were not detected by other presurgical diagnostic tests.

TABLE 1. *Monoclonal Antibody (MAb) Imaging Performance in Presurgical Patients Evaluated for Colorectal Carcinoma (n = 102)*

		Per Patient Analysis	
Patient Population	No. of Patients	% (No.) of Patients Correctly Diagnosed by MAb Imaging	Imaging Performance Parameter
Patients with confirmed adenocarcinoma	92	70% (64/92)	Sensitivity
Patients who were tumor-free at surgery	10	90% (9/10)	Specificity
All evaluable patients	102	72% (73/102)	Accuracy
		Per Lesion Analysis	
		No. of Lesions Detected by MAb Imaging	Imaging Performance Parameter
Lesion type			
Confirmed adenocarcinoma		82/126 (65%)	Sensitivity
Unconfirmed lesions		5	False positives

To delineate further the performance of ^{111}In-CYT-103 immunoscintigraphy in colorectal cancer patients, imaging performance in various subgroups of patients and imaging sensitivity for the detection of various subsets of lesions were examined (Table 2). The accuracy of immunoscintigraphy was similar in patients with primary and recurrent disease. The influence of previous adjuvant therapy on antibody imaging performance also was investigated. Eleven patients had received previous radiation therapy and 16 received previous chemotherapy. Most patients had discontinued these adjuvant therapies more than 1 year before ^{111}In-CYT-103 administration and none of the patients received antitumor treatments during the 4 weeks preceding infusion of the radiolabeled antibody conjugate. The data showed that antibody imaging performance in this subset of patients was similar to that in patients who did not receive previous adjuvant therapy.

In 10 patients with elevated CEA levels and negative presurgical workups, antibody imaging detected surgically confirmed occult lesions in four patients and confirmed the negative workup in three patients who were noted at surgery to have no evidence of tumor recurrence. In the remaining three patients, antibody imaging, as well as standard diagnostic procedures, failed to identify the surgically confirmed recurrence.

Antibody scan findings confirmed the absence of additional disease in 18 of 22 patients with isolated pelvic or liver recurrences. Importantly immunoscintigraphy detected extra-abdominal disease, including lesions in the lymph nodes (1), lung (3), bone (6), and brain (1), in 9 patients. Adenocarcinoma at these extra-abdominal sites was confirmed through biopsy of lung (2) and bone (1) in 3 of these patients. Brain (1) and/or bony lesions (3) initially identified by antibody imaging were detected by follow-up diagnostic tests in another 3 patients; the abnormal radiolocalizations noted for the 3 remaining patients were not explored surgically or examined by other imaging modalities.

Imaging sensitivity tended to be higher ($p = 0.10$) in patients with positive (85%) *versus* negative (63%) serum titers of TAG-72 (Table 2). The sensitivity of antibody imaging increased significantly ($p = 0.04$) with the TAG-

TABLE 2. *Influence of Various Factors on Antibody Imaging Performance*

Group/Parameter Evaluated	Antibody Imaging Performance
A. Status of patient's disease	
Primary disease	Accuracy = 74% (45/61)
Recurrent disease	Accuracy = 68% (28/41)
B. Patients with isolated recurrences in the pelvis or liver	Confirmed isolated disease in 82% (18/22)
C. Patients with elevated CEA levels and negative presurgical workups	Accuracy = 70% (7/10)
D. Prior adjuvant therapy?	
Yes	Accuracy = 70% (14/20)
No	Accuracy = 72% (59/82)
E. Preinfusion serum TAG-72 titers	
Positive (≥10 U/mL)	Sensitivity = 85% (17/20)
Negative	Sensitivity = 63% (39/62)
Not evaluated	Sensitivity = 80% (8/10)
F. Percentage of tumor cells that expressed TAG-72	
<5%	Sensitivity = 48% (10/21)
≥5% and <50%	Sensitivity = 65% (31/48)
≥50%	Sensitivity = 79% (15/19)
Not evaluated	Sensitivity = 68% (26/38)

CEA, carcinoembryonic antigen.

72 antigen content of the tumor. Although the sensitivity of immunoscintigraphy was somewhat lower for the small tumor lesions, the antibody scans detected 50% (6 of 12) of lesions measuring 2 cm or less. Furthermore no statistically significant correlation was detected between imaging sensitivity and lesion size.

Eighty-five of ninety-two patients with surgically confirmed colorectal carcinomas, the patient with small cell lung cancer, and 9 of the 10 patients who were tumor-free at surgery were evaluated by CT imaging as well as by immunoscintigraphy. In these patients CT imaging correctly identified tumor lesions in 66% (56 of 85) of patients with surgically confirmed adenocarcinoma, and CT scans were negative for 8 of 9 patients (89%) found to be tumor free at surgery. Thus CT imaging provided correct diagnoses for 64 of the 94 patients evaluated, for an overall accuracy rate of 68%. In addition a CT scan detected abdominal and chest disease in the patient with small cell carcinoma of the lung. In the 85 patients evaluated by both imaging modalities, the per-patient accuracy (69% *versus* 66%) and the overall per-lesion sensitivity (Table 3) of antibody and CT imaging were numerically similar. However immunoscintigraphy correctly identified tumor lesions in 17 patients with negative CT scans, and CT imaging detected tumor in 14 patients with negative antibody images (Fig. 1). Thus, although the populations for whom CT and antibody imaging detected surgically confirmed tumor lesions overlapped, they were not identical. Furthermore the aggregate findings of CT and antibody imaging correctly identified tumor lesions in 73 of the 85 patients (86%) who were subsequently found to have surgically confirmed adenocarcinoma.

Although the overall performance of CT and antibody imaging was similar, antibody imaging detected a significantly ($p = 0.02$) greater percentage of lesions in the pelvis and in other extrahepatic areas, whereas CT imaging detected a significantly ($p = 0.002$) greater proportion of liver lesions (Table 2).

Patient Management

The impact of antibody imaging on patient management was assessed in a subset of patients ($n = 69$) through the use of questionnaires completed before and after surgery. Based on the surgical and histopathologic findings, the investigators considered immunoscintigraphy to have been beneficial or very beneficial for 26% of the patients (Table 4). In these patients the antibody scans detected previously occult lesions, confirmed the existence of localized disease without regional or metastatic spread, indicated that abnormal findings on other diagnostic tests corresponded to adenocarcinoma, and made a diagnosis of recurrent colorectal cancer less likely in a patient with a second primary malignancy (small cell lung cancer). The antibody scans of two of these patients are provided in Figures 2 and 3. Immunoscintigraphy was considered negative or very negative in the management of two patients; in these cases the antibody scan failed to localize the primary tumor lesion of one patient and a false-positive finding in the liver on the other patient's scan led to the performance of a contrast CT of the abdomen and a liver–spleen scan, which failed to confirm the result.

Safety

All 116 patients enrolled in the trial were evaluated for safety. Seven patients (6%) experienced a total of nine adverse effects that were considered possibly or probably related to ^{111}In-CYT-103 administration. The most frequent adverse reactions, each experienced by three patients, included fevers and itching. In addition one patient each experienced mild hypertension, queasiness, and an-

TABLE 3. *A Comparison of the Detection Rates of Antibody and CT Imaging for Various Adenocarcinoma Lesions*

Imaging Modality	Sensitivity by Lesion Location			Overall Sensitivity
	Pelvis*	Liver†	Other*	
Antibody scans	74% (29/39)	54% (20/37)	68% (27/40)	66% (76/116)
CT scans	59% (23/39)	86% (32/37)	48% (19/40)	64% (74/116)

* Per lesion sensitivity of antibody imaging for disease located in the pelvis and other extrahepatic sites is significantly ($p = 0.02$) greater than that of CT imaging.

† Per lesion sensitivity of CT imaging for liver lesions is significantly ($p = 0.002$) greater than that of antibody imaging.

CT, computed tomograph.

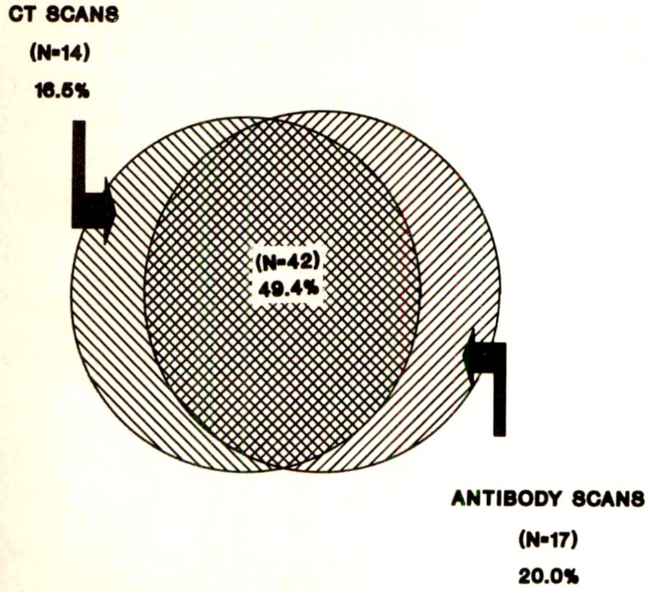

FIG. 1. A comparison of the populations of patients with surgically confirmed colorectal cancer who were correctly diagnosed by antibody and/or CT imaging. A total of 85 patients with adenocarcinoma were evaluated by both imaging methods.

gioedema. All of these adverse reactions resolved spontaneously or with appropriate treatment, and none of these signs or symptoms was considered serious. No clinically significant laboratory abnormalities related to the ^{111}In-CYT-103 infusions occurred during this study.

HAMA Response

Pre- and postinfusion serum samples were obtained from 95 of the 116 patients; these samples were evaluated for the presence of antibodies to murine immunoglobulin (HAMA). All patients were HAMA-negative before ^{111}In-CYT-103 administration, and 31 of the 95 patients (33%) developed positive HAMA titers after infusion.

Discussion

An ideal diagnostic approach for colorectal cancer would combine high sensitivity and specificity for primary or locally recurring colorectal tumors with the ability to detect accurately regional and distant metastases. This would include the detection of tumor deposits in lymph nodes and other soft tissues in the abdomen and retroperitoneal area that are commonly missed by currently available diagnostic tests.[2] Importantly any new modality must be able to influence the management of patients with colorectal cancer and compete with other diagnostic approaches in terms of relative cost and ease of use as well as sensitivity and specificity.[16] Such a procedure could be useful for the evaluation of both primary and recurrent disease patients. During the initial workup, this modality would be used for presurgical staging, thus assisting in planning and, potentially, in altering the operative approach. The identification of occult disease in patients who are scheduled for second-look surgery for suspected recurrence could help define the surgical procedure to be undertaken. Of special interest in this regard are patients with presumed isolated, resectable disease and patients with increasing serum CEA levels and negative presurgical workups.[17]

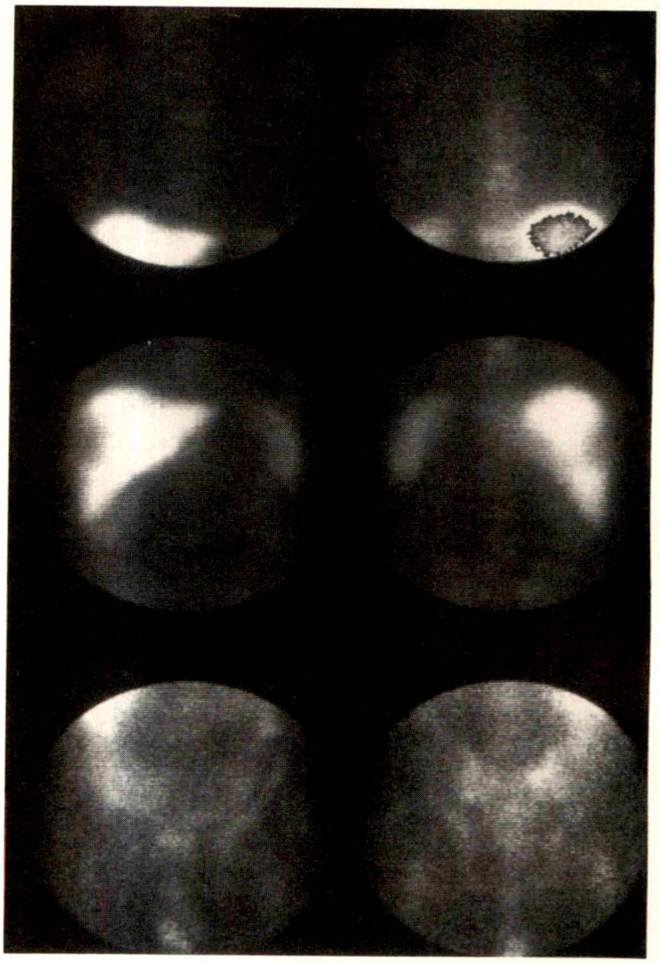

FIG. 2. Planar images, acquired 72 hours after ^{111}In-CYT-103 administration, of patient who presented with liver lesions and no known primary lesion. Antibody scan suggested colon primary, detected liver lesions, and also identified multiple bony metastases in the right shoulder, left posterior rib, and pelvis. Biopsy findings confirmed the presence of adenocarcinoma in liver and bone (rib). The previously scheduled placement of an intrahepatic artery catheter was not performed.

TABLE 4. *Investigators' Assessments of the Impact of Monoclonal Antibody (MAb) Imaging on Patient Management*

Effect of the MAb Scan Findings on Management of the Patient	No. (%) of Patients
Very beneficial	6/69 (9%)
Beneficial	12/69 (17%)
No effect	49/69 (71%)
Negative	1/69 (1%)
Very negative	1/69 (2%)

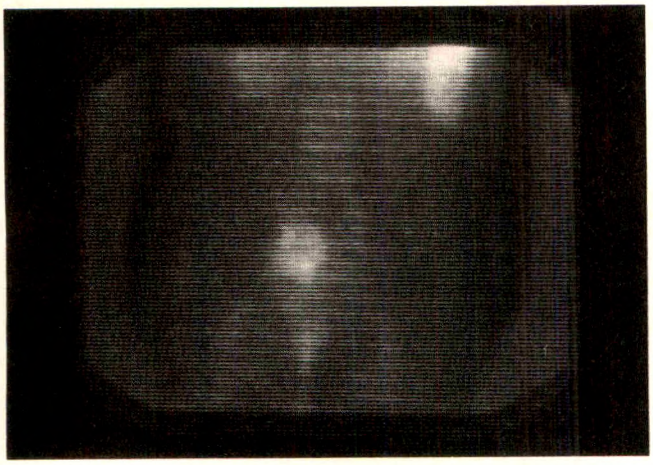

FIG. 3. Posterior planar images, acquired 48 hours after infusion, of patient evaluated for recurrence of colorectal adenocarcinoma. The antibody scan demonstrated that the pelvic mass detected by computed tomographic and magnetic resonance imaging represented a localized recurrence. These findings were confirmed at surgery.

The results of this multicenter clinical trial suggest that ^{111}In-CYT-103 immunoscintigraphy can provide information that is complementary to that derived from standard diagnostic modalities and can contribute beneficially to the management of presurgical patients with colorectal carcinoma. Beneficial patient management effects were noted for both primary and recurrent disease patients. In a previous report, the management benefits related to administration of an ^{111}In-labeled anticarcinoembryonic antigen-imaging agent were chiefly noted in patients with recurrent colorectal cancer.[17]

Importantly, antibody scans detected occult tumor lesions in 11 of 92 patients with surgically confirmed adenocarcinoma. Six were patients who were being evaluated for surgical removal of disease recurrence. The ability to assess more accurately the extent of disease in this patient population is important because a potentially curative procedure may be undertaken in recurrent disease patients with localized disease.[18,19] Alternatively the identification of unresectable disease may result in selection of more appropriate nonsurgical treatments, such as radiation or chemotherapy. Immunoscintigraphy also identified occult lesions in five patients with primary colorectal cancer. In these patients antibody imaging either detected more extensive disease or was the only presurgical test that correctly located the primary tumor. These findings could have a significant effect on surgical decision making, and these results suggest that the value of antibody imaging in the management of patients with colorectal carcinoma is not restricted to patients with recurrent disease.

^{111}In-CYT-103 immunoscintigraphy provided accurate diagnoses for 7 of 10 patients with elevated CEA levels and otherwise negative presurgical workups. In four of these patients, antibody imaging detected occult lesions and the antibody scan findings confirmed the negative workup in three patients with no evidence of recurrent disease at surgery. In the remaining three patients, immunoscintigraphy and standard diagnostic procedures failed to detect the surgically confirmed recurrence. Similar results have been reported using ^{111}In-labeled ZCE-025, a monoclonal antibody directed against CEA.[20] The authors reported that radiolabeled ZCE-025 provided accurate diagnoses in 12 of 13 colorectal cancer patients with elevated CEA levels and negative radiologic workups. Taken together these findings indicate that immunoscintigraphy with radiolabeled monoclonal antibodies may substantially contribute to the evaluation of patients with increasing CEA levels who are at high risk for recurrence of colorectal cancer and for whom a major operative procedure is contemplated.

The assessments of the patient management impact of ^{111}In-CYT-103 immunoscintigraphy corroborated the imaging performance findings for the study population and for various subgroups of patients. The antibody scan findings contributed beneficially to management decisions in 18 of 69 patients (26%) evaluated. Antibody scans contributed equally to the management of primary and recurrent disease patients. In general beneficial ratings were assigned in cases in which antibody imaging either detected previously occult lesions, which were later documented at surgery, or confirmed localized disease in patients scheduled for curative resections. In the two patients (3%) with negative patient management ratings, the antibody images failed to detect the primary tumor in one case and suggested additional imaging tests, which failed to confirm the abnormal radiolocalization, in the other.

Although the sensitivity and specificity of antibody imaging were similar to those of CT scanning, it is interesting to note that antibody imaging correctly identified tumor lesions in 17 patients with negative CT scans, and CT scans correctly identified tumor lesions in 14 patients with negative antibody images. In the population studied, CT was superior for detection of hepatic lesions, whereas immunoscintigraphy was superior for extrahepatic disease, including extra-abdominal lesions and those in the mid abdomen and in the pelvis, an area where CT imaging is purported to be helpful.[21] These results suggest that the use of a combination of these two presurgical diagnostic tests, each of which is particularly useful for evaluating certain anatomic regions, can provide complementary information and greater overall accuracy than either test used alone.

No serious clinical toxicity was observed in this multicenter trial. As noted one in three patients infused with ^{111}In-CYT-103 developed positive HAMA titers after infusion. In this single-infusion study, all patients were HAMA negative before infusion of the study agent; therefore the impact of circulating HAMA on the safety and efficacy of ^{111}In-CYT-103 was not evaluated. Published literature suggests that the clearance of monoclonal antibody-based products may be increased in patients with detectable levels of HAMA.[22] However preliminary results indicate that these agents can be readministered safely in the presence of circulating HAMA and that diagnostic images have been obtained following repeated administration.[23,24] A study is in progress to investigate repeated administration of ^{111}In-CYT-103 in patients with colorectal carcinoma.

The limited clinical toxicity observed for this agent combined with its demonstrated ability to contribute beneficially to patient management decisions through detection of primary and recurrent colorectal tumors, including occult lesions, indicate that ^{111}In-CYT-103 is a useful tool in the presurgical evaluation of colorectal cancer patients.

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The Prognosis of Melanoma Patients with Metastases to Two or More Lymph Node Areas

RICHARD J. BARTH, JR., M.D.,* DAVID J. VENZON, PH.D.,† and ALAN R. BAKER, M.D.*

From the Surgery Branch and the Biostatistics and Data Management Section,† National Cancer Institute, National Institutes of Health, Bethesda, Maryland*

The prognosis of melanoma patients who present with metastatic involvement of two or more noncontiguous lymph node regions before the detection of extranodal metastases has not been previously reported. We identified 21 patients with metastatic melanoma in at least two nodal basins in a review of 175 patients with melanoma undergoing lymphadenectomy at the National Cancer Institute. The median survival time of these patients was 46 months, with 55%, 27%, and 17% of the patients alive 2, 5, and 10 years, respectively, after the second lymphadenectomy. Because the prognosis of melanoma patients with metastases to two or more regional nodal areas appears equivalent to that of patients with metastatic involvement of only one regional node site, lymphadenectomy of the involved groups should be performed with therapeutically curative intent.

IT IS WELL established that the prognosis of melanoma patients with metastases only to a regional lymph node area is substantially better than the prognosis of patients with metastatic spread to extranodal sites.[1-16] Sometimes patients present with synchronous or metachronous metastatic involvement of two or more noncontiguous lymph node sites before the appearance of extranodal disease. The prognosis of these patients has not, to our knowledge, been previously reported.

The American Joint Committee on Cancer (AJCC) classification of these patients by stage, summarized in Table 1, has been in a state of flux. In 1983 patients with involvement of more than one regional lymph node site were classified as having stage IV disease, thereby equating their prognosis to that of patients with extranodal metastases.[17] Intuitively, however, one might surmise that a primary melanoma located between two regional nodal areas might drain to both of them; therefore the presence of metastatic melanoma in both of these regional areas may not necessarily predict a worse prognosis than the same number of metastases in one nodal region. Intuition might further suggest that if one of the two involved lymph node sites were outside the regional drainage area of the primary, the prognosis might reflect that of a more widely metastatic process. The most recent (1988) AJCC staging system integrates these intuitions: patients with regional nodal metastases, regardless of the number of nodal areas involved, are classified as stage III, while patients with distant nodal metastases are considered to be stage IV.[18] Because the therapeutic approach to such patients is influenced greatly by their prognosis and there is little data in the literature to prognostically validate either staging system, we reviewed the clinical course of melanoma patients with metastases to two or more lymph node areas treated at the National Cancer Institute (NCI) to determine the frequency of this occurrence and its clinical outcome.

Methods

A computer search performed on the NCI Clinical Center medical record department files identified 175 patients with melanoma who underwent lymphadenectomy between the years 1960 and 1987. Thirty-eight of these one hundred seventy-five patients had two or more lymph node regions dissected. The charts of these 38 patients were analyzed in detail.

For the purposes of this analysis, the neck, axilla, and inguinal lymph node areas were considered to be separate nodal regions because a primary melanoma located between these regions theoretically could metastasize sep-

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Address reprint requests to R. J. Barth, Jr., Room 2B46, Bldg. 10, Surgery Branch, National Cancer Institute, Bethesda, MD 20892.

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TABLE 1. *American Joint Committee on Cancer Staging System for Melanoma Patients with Advanced Regional Nodal Disease*

Year	Stage III	Stage IV
1983 Version	Any T, N1 (involvement of only one regional lymph node station; node(s) movable and not larger than 5 cm in diameter, or negative regional lymph nodes and the presence of less than five in-transit metastases beyond 2 cm from the primary site), M0.	Any T, N2 (any of the following: (1) involvement of more than one regional lymph node station; (2) regional node(s) larger than 5 cm in diameter or fixed; (3) five or more in-transit metastases beyond 2 cm from the primary site with regional lymph node involvement), M0; or any T, any N, M1.
1988 Version	Any T, N1 (metastasis 3 cm or smaller in greatest dimension in any regional lymph node(s) or N2 (metastasis larger than 3 cm in greatest dimension in any regional lymph node(s) and/or in-transit metastses), M0.	Any T, any N, M1 (metastasis in skin or subcutaneous tissue or lymph node(s) beyond the regional lymph nodes or visceral metastasis).

arately to each area. Lymph nodes were considered to be regional drainage areas according to conventional criteria as defined in the *1988 Manual for Staging of Cancer.*[18] The superficial and deep inguinal nodes were not considered to be two separate node sites. Patients who had lymph node biopsies only were excluded.

All patients were followed in the Surgery Branch, NCI until the detection of extranodal melanoma metastases. Many patients with disseminated disease continued to be followed and treated at the NCI. Follow-up information on patients referred elsewhere for treatment was obtained by telephone contact.

Survival curves were generated using the method of Kaplan and Meier.[19] Duration of survival was calculated from the second lymphadenectomy unless otherwise stated. Statistical comparisons were made using the log rank test with two-tailed probability values.

Results

One hundred seventy-five patients with melanoma had at least one lymph node dissection performed at the NCI between the years 1960 and 1987. Of these 38 patients had dissections of two different lymph node regions before evidence of extranodal metastatic disease appeared. In five of these 38 patients, both dissections were histologically negative for melanoma; in 12 patients only one of the two dissections revealed metastatic melanoma. In the remaining 21 patients (12% of 175), histologic evaluation of the lymphadenectomy specimens demonstrated metastases in at least two different lymph node regions.

These 21 patients, with histologically confirmed metastases to at least two lymph node areas before extranodal metastases were detected, were studied in detail. Their clinical characteristics are summarized in Table 2. There were 18 male and 3 female patients. Their ages ranged from 17 to 63 years, with a median of 33 years. Sixteen patients had their primary melanoma located on the trunk, two patients had an extremity primary, and three were assigned to the 'unknown primary lesion' category. The Clark level of the primary lesion, and the number of patients with invasion to that level were as follows: level I, 0; II, 3; III, 7; IV, 5; V, 2; not described, 1.

The sizes of the primary lesions and the locations of the lymph node areas dissected are presented in Table 3. Of the 18 patients with a primary tumor whose location was known, 14 had nodal dissections at sites that could reasonably be considered to be regional drainage basins. Four patients (numbers 15, 17, 19, and 20 [Table 3]) had lymphadenectomies that revealed metastatic involvement of distant lymph node areas. Four patients (numbers 4, 14, 15, and 21 [Table 3]) had a third node dissection done before extranodal metastases were detected.

Lymph node dissections were done for palpable masses with therapeutic intent in all cases except one; in this instance there was inadequate documentation in the chart to determine intent. The time interval between the two lymph node dissections performed ranged from 0 to 27 months. In 12 patients the dissections were done within

TABLE 2. *Clinical Characteristics of Melanoma Patients with Metastases to at Least Two Lymph Node Areas*

Clinical Characteristics	Number of Patients
Sex	
Male	18
Female	3
Age (yrs)	
Range	17–63
Median	33
Location of primary lesion	
Trunk	16
Extremity	2
Unknown	3
Clark level of primary lesion	
I	0
II	3
III	7
IV	5
V	2
Not described	1
Interval between node dissections	
≤1 month	12
>1 month	9
Number of positive lymph nodes per patient	
<4	8
≥4	13

TABLE 3. *Location of Primary Melanoma, Lymphadenectomy Site, and Patient Survival*

Patient (n)	Primary Site	LN Diss #1	LN Diss #2	LN Diss #3	Survival (months)*
1	Mid back	L axilla	R axilla		185
2	R upper chest	R axilla	R neck		162+
3	L low abdomen	L groin	R groin		113+
4	Mid back	L axilla	R axilla	R groin	100
5	Unknown	L axilla	R axilla		55
6	Unknown	L groin	R groin		49+
7	L flank	L groin	L axilla		49
8	L low back	R groin	L groin		48
9	Scalp	R neck	L neck		44
10	R shoulder	R axilla	R neck		38
11	Mid back	R axilla	L axilla		36+
12	Unknown	R axilla	R neck		20
13	L shoulder	L axilla	L neck		17
14	Posterior neck	R neck	R axilla	L neck	16
15	L low back	R axilla	L axilla	L groin	14
16	R submental	R neck	L neck		13
17	R flank	R axilla	L axilla		12+
18	L low back	L axilla	L groin		10
19	R low back	R axilla	L axilla		10
20	R upper abdomen	R axilla	R neck		6
21	Mid chest	L axilla	L neck	R axilla	4

* Measured from the second lymphadenectomy.
LN Diss, lymph node dissection.

1 month of each other, while the lymphadenectomies in the remaining nine patients were done a median of 9 months apart (Table 2). The combined number of histologically positive lymph nodes found in the two dissected groups ranged from 2 to 35 per patient; 62% of patients harbored a total of four or more lymph nodes containing metastatic melanoma (Table 2).

Within the context of randomized prospective treatment trials, 13 patients received adjuvant therapy after recovery from their first or second lymph node dissection. Four patients received adjuvant chemotherapy with single-agent dacarbazine (DTIC) or methyl cyclohexylnitrosourea (CCNU). Nine patients received adjuvant immunotherapy with bacillus Calmette-Guérin (BCG) +/− an allogeneic tumor cell vaccine. None of these adjuvant regimens was demonstrated to be beneficial.[20]

Once extranodal metastases developed, 10 patients underwent further surgery to palliate symptoms. Five patients were treated with various chemotherapeutic regimens and one patient received intralesional BCG for extensive cutaneous metastases. No patient received an interleukin-2 (IL-2)-based immunotherapy regimen.

The overall survival of the 21 patients with at least two pathologically positive lymph node dissections, measured from the time of the second node dissection, is summarized in Table 3 and Figure 1. The median survival was 46 months while survival at 2, 5, and 10 years was 55%, 27%, and 17%, respectively.

Albeit anecdotal, prolonged survival time of patients with metastases to three regional lymph node sites was noted. Overall survival time of the four patients (numbers 4, 14, 15, and 21 [Table 3]) who underwent dissections of three different nodal groups harboring metastases was 4, 13, 14, and 91 months, respectively, after their third lymph node dissection.

Metastases to nonregional lymph node sites in the absence of extranodal dissemination are rare; only four patients (numbers 15, 17, 19, and 20 [Table 3]) were identified with such in this study. The overall survival time of these four patients was 6, 10, 12+, and 14 months, respectively.

To determine whether the survival of patients with

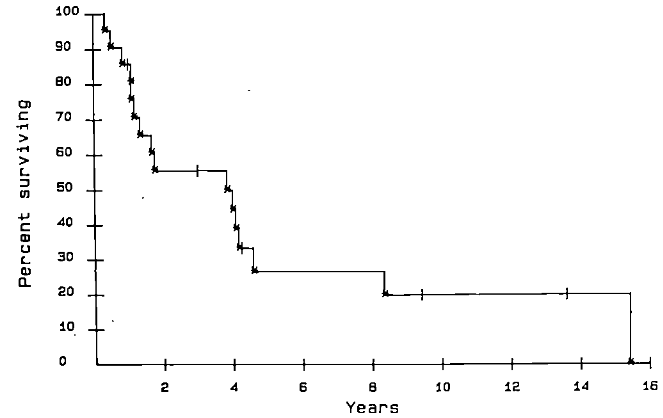

FIG. 1. Overall survival of melanoma patients undergoing dissections of two or more histologically positive lymph node sites before the appearance of extranodal metastases. Survival is measured from the time of the second lymphadenectomy.

TABLE 4. *Overall Survival after Therapeutic Lymphadenectomy for Melanoma*

Published Study	No. of Patients	% Clinical Stage III	Median (months)	Survival (years) 2(%)	5(%)	10(%)
1. Calabro, MD Anderson 1989	1001	93			33	28
2. Balch, UAB 1980	185	100			28	15
3. Karakousis, RPMI 1980	125	90	26	57	28	
4. Jones, Cardiff 1968	22	90			18	
5. McNeer, MSKCC 1964	295	100			19	12
6. Goldsmith, MSKCC 1970	406	100			38	
7. Callery, UCLA 1982	150	80		55	37	34
8. Cohen, NCI 1977	57	100				38
9. Roses, NYU 1985	56	100			20	12
Total	2297	Average:	26	56	28	23
Barth, NCI 1990						
Total	21	Average:	46	55	27	17

synchronous metastatic involvement of two lymph node areas differed from that of patients with metachronous metastases, the survival of patients whose nodal dissections were done month or less apart (n = 12) was compared to that of patients whose dissections were done more than 1 month apart (n = 9). As shown in Figure 2, there is no statistically significant difference (p = 0.62) in the survival of patients with synchronous or metachronous involvement of a second nodal group.

The survival of patients with a total of four or more positive lymph nodes (n = 13) was compared with the survival of patients with three or fewer positive lymph nodes (n = 8). Log rank analysis of these Kaplan–Meier survival curves revealed no significant survival difference (p = 0.90).

Discussion

We investigated the clinical course of patients burdened by two or more lymph node areas involved with melanoma before extranodal disease was detected. Our identification of 21 such patients, of a group of 175 melanoma patients treated with lymphadenectomy at the NCI, suggests that this is not an uncommon clinical problem.

Previous reports have analyzed the significance of multiple variables on the prognosis of patients undergoing lymph node dissection for melanoma. Many studies have concluded that increasing numbers of histologically positive regional lymph nodes portend a progressively poorer prognosis.[2–4,8,9] Other variables that may indicate a poor prognosis include therapeutic dissection for palpable nodes (*versus* prophylactic dissection of nodal regions),[9,10] axial location of the primary lesion,[9] ulceration of the primary lesion,[3,8] and increasing thickness of the primary lesion.[8]

Examination of the characteristics of our study population of 21 patients with two nodal areas containing metastases reveals a preponderance of poor prognostic indicators A high percentage of patients (62%) had a total of four or more histologically positive lymph nodes in the two dissected groups. All patients had therapeutic dissections for palpable disease. Sixteen of the eighteen primary lesions whose location was known were excised from an axial site. There was insufficient documentation of the presence of ulceration and Breslow thickness in our study population to consider these variables. However 14 of the 18 primary melanomas had a Clark level of invasion described as III or higher. Based on these characteristics of the study population, therefore, one would predict a poorer survival rate when compared to other groups of patients undergoing lymphadenectomy for metastatic melanoma.

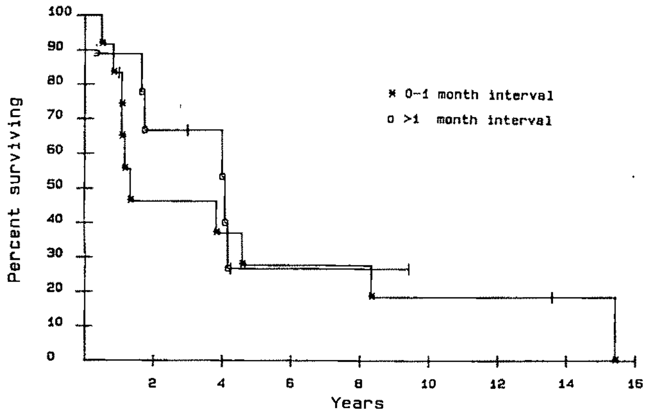

FIG. 2. Effect of the interval between dissections on the overall survival of melanoma patients undergoing dissections of two histologically positive lymph node sites. Survival is measured from the time of the second lymphadenectomy.

TABLE 5. *Overall Survival from Diagnosis of Metastatic (Stage IV) Melanoma*

Published Study	No. of Patients	Median (months)	Survival (yrs) 2 (%)	Survival (yrs) 5 (%)
1. Presant, UAB 1982	277	6		
2. Stehlin, MD Anderson 1966	222		6	
3. Einhorn, MD Anderson 1974	426	5		
4. Balch, UAB 1983	200	6	5	
5. Amer, Wayne State 1979	140	6		
6. Hena, RPMI 1987	180	8		5
Total	1445	Average: 6	6	5
Barth, NCI 1990	21	Average: 46	55	27

In an attempt to obtain a reasonable approximation of the survival of patients undergoing therapeutic lymphadenectomy for metastatic melanoma, the results of nine large studies were compiled (Table 4). Similarly to obtain an approximation of the survival of patients presenting with extranodal metastatic melanoma, six large series were examined (Table 5). The approximate median as well as 2-, 5-, and 10-year survival rates of these two groups are compared with our own study population at the bottom of Tables 4 and 5. It is evident from these comparisons that the prognosis of our patients with two or more regional nodal areas containing metastases appears equivalent to that of reported patients with metastases to only one regional lymph node area. Furthermore the prognosis of our patients with two positive nodal dissections appears substantially better than that of reported patients with extranodal metastatic disease.

Although some patients received adjuvant methyl CCNU chemotherapy or BCG +/−, an allogeneic tumor cell vaccine immunotherapy, a randomized prospective trial of these therapies at the NCI failed to demonstrate any survival benefit.[20] Similarly there is no evidence to suggest that the palliative operative procedures or chemotherapy administered to some of these patients, once extranodal metastases were detected, had a substantial impact on survival.[13,16]

Four patients (numbers 15, 17, 19, and 20 [Table 3]) had metastases to nonregional lymph node basins. Although their survival time appears to be worse (6, 10, 12+, and 14 months) than the rest of the group, the only conclusion that can be made about this subset is that metastases to distant lymph nodes in the absence of extranodal metastatic disease is an unusual occurrence (4 of 175 (2.3%) melanoma patients undergoing lymphadenectomy).

This study does not address the prognosis of melanoma patients who might metastasize from one lymph node region to a second more proximal group of contiguous lymph nodes because there is only one patient (number 20 [Table 3]) who may have had this type of lymphatic spread. The clinical course of such patients has been described elsewhere. Melanoma patients with iliac and superficial inguinal nodal metastases appear to have a worse prognosis than patients with superficial inguinal disease alone.[21,22] In contrast the survival of patients with epitrochlear and axillary node involvement from upper extremity primary lesions was comparable to those with axillary involvement alone.[23]

Our observation that the prognosis of patients with metastatic involvement of two or more nodal areas appears equivalent to that of patients with metastatic involvement of only one regional nodal area suggests the following clinical strategy.

1. If a melanoma patient presents with palpable masses in two regional nodal areas, these nodal areas should be dissected with therapeutic intent. If after initial lymphadenectomy a palpable mass develops in a second nodal area, it too should be dissected with curative intent.
2. A melanoma patient with metastases to two or more regional nodal groups should be prognostically classified as a stage III patient, as reflected in the most recent (1988) *AJCC Manual for Staging of Cancer.*

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Metabolic Consequences of (Regional) Total Pancreatectomy

CAROLYN M. DRESLER, M.D.,* JOSEPH G. FORTNER, M.D.,* KATHERINE McDERMOTT, R.N.,*
and DAIVA R. BAJORUNAS, M.D.†

From the Departments of Surgery and Medicine,† Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, New York*

Little information has been reported on the metabolic characteristics of the totally pancreatectomized patient or the efficacy of medical management after radical pancreatic surgery. The prospective evaluation of 49 such patients, with 31% followed for 48 or more months, forms the basis of this report. The major immediate postoperative challenge is control of diarrhea and weight stabilization. Chronically patients have an increased daily caloric requirement (mean ± SE, 56 ± 1 kcal/kg), not wholly explained by moderate steatorrhea (fecal fat excretion, 16% ± 2% of unrestricted fat intake). Despite persistent malabsorption, deficiencies in fat-soluble vitamin, magnesium, and trace element serum levels can be prevented in most patients. Pancreatogenic diabetes is characterized by (1) absence of the major glucoregulatory hormones insulin and glucagon, (2) instability, and (3) frequent hypoglycemia, with the latter parameters improving with rigorous home glucose monitoring. No patient has developed clinically overt diabetic micro- or macrovascular disease. Performance status in long-term survivors has been reasonable. However adverse chronic sequelae of the operation occur and include an unusual frequency of liver disease, characterized by accelerated fatty infiltration, and osteopenia, with an 18% reduction in radial bone mineral content noted in pancreatectomized patients studied more than 5 years after surgery.

THE FIRST TOTAL PANCREATECTOMY in humans was performed in 1942.[1] The value of this procedure in treating a variety of tumors of the pancreas has been evaluated in many reports in the intervening 47 years.[2-12] Little information, however, has been reported on the metabolic characteristics of the totally pancreatectomized patient or the efficacy of medical management. The diabetic state is often characterized as 'brittle.' It is assumed that the diabetes is otherwise similar to the spontaneously occurring disorder in adults, requiring similar management. Pancreatic exocrine deficiency has been studied as it relates to steatorrhea but with limited information on other nutritional deficiencies. Particularly striking is the absence of information on the long-term consequences of the operation.

This report concerns the prospective evaluation of 49 patients who had a total pancreatectomy as part of a resection that included a regional lymph node dissection, sympathectomy, and segmental portal vein resection with reconstruction (regional total pancreatectomy). Acute and chronic indices of malabsorption are reported. The diabetic state is characterized and its management discussed. Adverse chronic sequelae of the operation include an unusual frequency of liver disease and osteopenia; noteworthy are the absence of clinically overt diabetic micro- and macrovascular disease and the reasonable performance status in long-term survivors.

Materials and Methods

Patient Characteristics

The study population consists of all patients who underwent a complete pancreatic resection at Memorial Sloan-Kettering Cancer Center by one surgeon (JG Fortner) during the period January 1978 to June 1988 (n = 45). Four additional patients who had the procedure earlier (1972, 1975, 1975, 1976) are included as well. The 28 men and 21 women were 54 ± 2 years in age (mean ± SE; range, 29 to 72 years) at time of surgery.

Two patients had a standard total pancreatic resection.

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Address reprint requests to Daiva R. Bajorunas, M.D., Division of Endocrinology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021.

Dr. Dresler's current address is Department of Surgery, Washington University School of Medicine, St. Louis, MO 63110.

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The other 47 patients had a regional total pancreatectomy, which included a complete pancreatectomy, subtotal gastrectomy, duodenectomy, splenectomy, cholecystectomy with removal of the common bile duct, and regional lymph node dissection with accompanying resection of the celiac and superior mesenteric ganglia and removal of lymphatic and nerve tissues surrounding the superior mesenteric artery. A vagotomy was not done. The pancreatic portion of the portal vein was removed *en bloc* and reconstructed with an end-to-end anastomosis of the superior mesenteric vein to the portal vein. Details of the surgical procedure have been previously described.[13,14] Table 1 depicts the diagnoses prompting the surgical procedures.

TABLE 1. *Indications for Surgery*

Histologic Diagnoses	No. of Patients
Adenocarcinoma of the pancreas	31
Periampullary carcinoma	7
Islet cell carcinoma	2
Papillocystic adenocarcinoma	1
Pancreatitis	7
Lymphoma	1

Management Protocol

A team approach to patient management was used, with all patients followed by the same surgical and endocrine attending physicians (JG Fortner and DR Bajorunas) for the duration of the 11-year follow-up period. The team nurse clinician provided patient education on a daily basis during the initial hospitalization and was present at every outpatient visit.

Dietary guidelines. After operation all patients were managed for up to 3 days in the intensive care unit and after their transfer to the general surgical floor, total parenteral nutrition (TPN) was maintained until adequate oral feedings were clinically feasible. Thereafter the patients were placed on a high calorie (40 to 50 kcal/kg), three-meal, three-snack diet/day, limited in sucrose calories only if the patients were maintaining weight. Additional caloric intake was encouraged by the use of lactose-free, lower osmolarity oral formula supplements and the liberal use of medium-chain triglyceride (MCT) oil. No specific dietary fat restriction was placed and the overall caloric distribution approximated 35% to 40% fat, 45% to 50% carbohydrate, and 15% to 20% protein calorie intake. In patients whose diarrhea was protracted, an empiric lactose-free diet was instituted.

Patients were discharged from the hospital only when a trend toward weight stabilization was demonstrated and calorie counts showed an intake of at least 2000 kcal/day. Following discharge all patients were maintained on the same dietary guidelines that focused on calorie-dense foods, except that a simple sugar restriction (less than 5% sucrose calories) was introduced to aid glycemic control when dietary intake had stabilized. A registered dietitian evaluated the patients every 3 months for the first postoperative year and yearly thereafter.

Diabetic management. The patients were instructed in the use of a reflectance meter-assisted home glucose monitoring program[15,16] before their discharge from the hospital. Comparable mean values to laboratory glucose determinations were achieved ($r = 0.85$, $p = 0.001$) when the patients were monitored as inpatients. Fingerstick glucose determinations were obtained before meals and at bedtime; in the immediate posthospital discharge period and periodically thereafter, patients were asked to check a 3 A.M. level. Compliance with this regimen on a long-term basis was readily achieved because both patients and their families thought that this technique provided them with increased security from hypoglycemia. At every subsequent hospital outpatient visit these glycemic records were reviewed with the patients and their families, and insulin dosage adjustments were instituted.

The patients were best managed on a regimen of 2:1 or 3:1 ratio of NPH/Lente:regular insulin administered subcutaneously in the morning, with an additional dose of regular insulin administered 30 minutes before dinner. Because of the frequency of nocturnal hypoglycemia, only the exceptional patient required a second (evening) injection of intermediate-acting insulin. All regular insulin was administered per a sliding scale regimen, depending on the result of the fingerstick glucose determination. A 'salvage' regular insulin sliding scale was prescribed to prevent excessive hyperglycemia before lunch and at bedtime. All patients were strongly encouraged to maintain a bedtime fingerstick glucose level of more than 11.2 mmol/L (200 mg/dL). Patients and family were well versed in the signs and symptoms of hypoglycemia, and glucagon (in 1 mg/mL vials) for parenteral administration by family members in the event of a serious hypoglycemic episode was routinely prescribed.

Management of pancreatic exocrine deficiency. The patients were instructed to take pancreatic enzyme replacement, consisting of variable amounts of lipase, amylase, and protease units depending on the commercial preparation used, as soon as oral intake was instituted. Doses were slowly increased to a usual maintenance dose of four to five capsules with meals and two to three capsules with snacks. The dosages of the enzymes were clinically adjusted on the basis of weight, serum magnesium levels, and stool characteristics. Antacids or H-2 blockers were not routinely prescribed. Enteric-coated microsphere formulations appeared to offer no specific advantage in this patient population. It was not unusual for tachyphylaxis to develop to a specific enzyme preparation, requiring a product change. Patients were encouraged to take two multivitamins/day and were prescribed oral calcium (1 g) and pharmacologic vitamin D (4000 to 12,000 IU) supplementation daily.

Laboratory Analyses

Routine hematology, chemistry screening profiles, and magnesium levels were obtained at every visit (bimonthly for the first 6 months, then at 3- to 6-month intervals). Glycosylated hemoglobin levels[17] were used to ascertain the degree of diabetic control. Vitamins A,[18] E,[19] 25-hydroxy, and 1,25-dihydroxy D,[20,21] and the trace elements copper and zinc[22] were monitored every 6 months. Measurement of bone mineral content used direct photon absorptiometry technique at the distal third of the nondominant radius.[23]

For the quantitative 72-hour fecal fat analyses,[24] patients were admitted to the metabolic unit and maintained on a defined diet that mimicked their outpatient dietary regimen. The usual pancreatic enzyme replacement was maintained. Nonabsorbable stool markers ensured adequacy of the 72-hour stool collection. Such inpatient testing validating outpatient management was supported by the close reproducibility of results in the four patients in whom such testing was repeated after at least 18 months. Carbohydrate absorption testing used an oral 25-g dose of D-xylose, with serum levels and urinary excretion measured during the subsequent 5 hours.[25]

In selected patients the completeness of the pancreatic resection was verified by stimulated C-peptide levels.[26] Plasma immunoreactive glucagon (IRG) and IRG chromatographic profiles were assayed in the laboratory of Dr. Jonathan Jaspan, University of Chicago, using a double antibody radioimmunoassay technique with 30K antiserum.[13] In this assay cross-reactivity with glucagonlike immunoreactivity is minimal. Glucagon chromatographic profiles were obtained with a 1 × 50-cm BioGel P-30 column (Bio-Rad Laboratories, Richmond, CA), as previously described.[27,28]

Data Analysis

All data obtained through December 1988 was analyzed. Survival data are presented for all patients who had their procedure during the study period (n = 49). Metabolic parameters are presented for 35 of these patients. The data is expressed for every patient as the mean of all determinations obtained. The studies were begun no earlier than 6 months after the operation to avoid the influence of any postoperative surgical complications. Laboratory data are not included for terminally ill patients. However the final chemistry screening profile in four patients was obtained within 3 months of death from recurrent disease. All results are expressed as mean ± SE, unless otherwise stated.

Results

Surgical Outcome

The mean (±SE) postoperative follow-up for all patients has been 39 ± 6 months (median, 15 months; range, 1 to 174 months), with 31% of patients followed for 48 or more months. The seven patients who were operated on for benign disease (chronic pancreatitis) have had a mean survival time of 80 ± 24 months (median, 86 months; range, 1 to 174 months). There was one perioperative death due to intestinal ischemia. Three patients died 116 ± 29 months after surgery of cirrhosis of the liver (n = 2) and a cerebrovascular accident (n = 1). Three other patients are alive and well 12, 60, and 139 months after pancreatic resection.

The mean survival for the 7 patients with periampullary carcinoma was 70 ± 19 months (median, 102 months; range, 6 to 117 months). Three patients died of recurrent disease within 27 months of surgery, and two others died from non-neoplastic causes (hepatic/renal failure, cirrhosis) 102 and 110 months after surgery, respectively. Two patients are alive and well at 113 and 117 months.

The 31 patients with pancreatic adenocarcinoma have had a mean survival time of 17 ± 4 months (median, 13 months; range, 1 to 88 months). There were three perioperative deaths in this population. Eight other patients died of causes unrelated to their cancer diagnosis at 35 days (hypoglycemia), 5 weeks (suicide), 6 weeks (gastrointestinal bleeding), 2 months (cerebrovascular accident), 4 months (sepsis), 6 months (sepsis), 8 months (pulmonary embolus), and 16 months (undetermined cause) after surgery. Fourteen patients died of recurrent disease 14 ± 2 months (median survival, 14 months) after pancreatic resection. Six patients are alive without evidence of disease 6, 24, 25, 48, 83, and 88 months after their pancreatectomy. The survival experience in the larger number of patients with adenocarcinoma of the head of the pancreas undergoing a curative total or subtotal regional pancreatectomy is the subject of a recent review.[29]

The mean follow-up for the 35 patients surviving for more than 6 months after surgery is 53 ± 8 months (median, 26 months; range, 8 to 174 months). Of these 89% have been followed for more than 1 year and 37% have been followed for more than 5 years.

Nutritional Status

The mean preillness weight of the study patients was 118% ± 3% of their ideal body weight (IBW). One half of the patients were obese, as defined as a preillness weight more than 120% IBW. For all patients the mean weight loss before their pancreatic resection was 8 ± 2 kg.

Prior to 1978, before intensive postoperative nutritional support was instituted, the mean perioperative weight loss was 15.9 ± 3 kg. Following that time aggressive hyperalimentation was routinely administered, which resulted in a perioperative weight loss of 4.8 ± 0.8 kg. The mean duration of TPN was 19 ± 4 days/patient, because anorexia, early satiety, diminished gastric capacity, delayed gastric emptying, and persistent and troublesome diarrhea seriously limited early tolerance to oral feeding. In four patients adequate oral intake was not achieved during a prolonged period of inpatient observation, and they were supplemented by an intermittent (12 to 16 hours per 24 hours) pump infusion of a defined-formula diet, which provided hydrolyzed protein, low residue, and no lactose, and which was administered *via* nasogastric feeding tube on an outpatient basis until oral intake and weight stabilized.

All patients experienced severe and debilitating diarrhea during the immediate postoperative period, which responded variably to conventional antidiarrheal agents. In most patients watery diarrhea abated within 3 to 12 months, although formed bowel movements remained frequent (4 to 8 per day), urgent, and bulky. Approximately 10% of patients, however, have continued to have significant and troublesome chronic diarrhea. Other digestive disturbances in patients free of recurrent disease were distinctly unusual. No patient noted abdominal pain as a complication of the surgical procedure. Nausea, vomiting, and dumping were not experienced after the immediate postoperative period. All long-term survivors thought that their meal capacity exceeded their prepancreatectomy pattern.

TABLE 2. *Selected Serum Indices of Liver Function**

Indices	Patient Values	Normal Levels
Total Bilirubin		
μmol/L	15 ± 2	2–18
(mg/dL)	(0.9 ± 0.1)	(0.1–1.0)
Total Protein		
g/L	70 ± 2	63–81
(g/dL)	(7.0 ± 0.2)	(6.3–8.1)
Albumin		
g/L	39 ± 1	40–52
(g/dL)	(3.9 ± 0.1)	(4.0–5.2)
SGOT		
μkat/L	0.48 ± 0.27	0.01–0.2
(U/L)	(60 ± 34)	(0–25)
Alkaline Phosphatase		
μkat/L	5.6 ± 1.0	0.5–1.9
(U/L)	(338 ± 59)	(30–115)

* Data obtained at the time of every follow-up visit. All values (mean/patient) expressed as mean ± SE for the entire patient group. Common unit values are provided in parentheses.

SGOT, serum glutamic oxaloacetic transaminase.

TABLE 3. *Seventy-two-Hour Fecal Fat Excretion** †

Dietary Fat mmol/day (g/day)	Fecal Fat mmol/day (g/day)	% Fat Excreted	Units Lipase/mmol (g) Fat Ingested
527 ± 35	84 ± 11	16 ± 2	179 ± 22
(150 ± 10)	(24 ± 3)		(630 ± 77)

* Results expressed as mean ± SE, with common unit values in parentheses. Normal fecal fat excretion is <4% of a measured fat intake in a 3-day period, or <21 mmol/day (6 g/day).

† Fifteen patients, 20 studies.

For the patients as a whole, the outpatient weight at last follow-up was 99% ± 4% IBW. Of the patients who survived more than 18 months after their pancreatic resection, the mean weight change from their hospital discharge weight was +2.2 ± 2.2 kg. In seven weight-maintaining patients, adherence to the recommended high-calorie diet was assessed by a review of food diaries and recall histories, and 3-day food records were submitted for computer analysis to assess nutritional soundness as well as the actual composition of the diets ingested. Caloric intake ranged between 2500 and 5300 kcal/day (mean, 56 ± 1 kcal/kg; range, 36 to 74 kcal/kg), was identical to the distribution prescribed, and was not deficient in any essential nutrient.

Selected parameters of liver function for all patients are depicted in Table 2. Elevated levels of serum glutamic-oxaloacetic transaminase (SGOT/AST) and alkaline phosphatase were seen in virtually all the patients. Total protein levels were normal in all patients, but in one half of the patients serum albumin levels remained persistently decreased.

Malabsorption

Nine patients were studied for their ability to absorb carbohydrates by the standard D-xylose test. The mean urinary 5-hour excretion of D-xylose was 6.5 ± 0.7 g, with a range of 3.5 to 10.7 g. All but one patient demonstrated normal carbohydrate absorption.

Table 3 depicts the 72-hour fecal fat excretion data obtained in 15 totally pancreatectomized patients. As noted, on a diet unrestricted in fat calories, the mean 24-hour fecal fat excretion was 16% ± 2% of dietary fat intake. No correlation could be found between the amount of dietary fat ingested and the degree of steatorrhea noted (r = 0.13).

Mean serum fat-soluble vitamin, magnesium, and trace element levels were within the normal range (Table 4). However persistently low values could be demonstrated in individual patients: abnormal levels of vitamin A (n = 4 patients), vitamin E (n = 1), 25-hydroxyvitamin D (n = 4), 1,25-dihydroxyvitamin D (n = 3), magnesium

TABLE 4. *Selected Nutritional Indices**

Indices	Patient Values	Normal Levels
Vitamin A		
IU/L	1090 ± 170	650–2750
Vitamin E		
mg/L	8.8 ± 3.3	5–20
25-hydroxyvitamin D		
nmol/L	67 ± 30	25–137
(ng/mL)	(27 ± 12)	(10–55)
1,25-dihydroxyvitamin D		
ng/L	25 ± 2	20–76
Magnesium		
mmol/L	0.78 ± 0.02	0.70–1.10
(mEq/L)	(1.55 ± 0.03)	(1.4–2.2)
Copper		
μmol/L	19.8 ± 0.9	11.0–24.4
(mcg/dL)	(126 ± 6)	(70–155)
Zinc		
μmol/L	13.5 ± 1.4	7.7–22.9
(mcg/dL)	(88 ± 9)	(50–150)

* Data obtained every 6 months. All serum values (mean/patient) expressed as mean ± standard error for the entire patient group, with common unit values in parentheses.

(n = 4), and zinc (n = 1) were noted, despite multivitamin and pharmacologic vitamin D supplementation in all patients. The prothrombin time was normal in all patients except in whom severe hepatic insufficiency supervened, and no patient required supplemental vitamin K administration.

While the 24-hour urinary calcium excretion was normal in all patients, mean serum calcium levels were subnormal in nine patients. Furthermore the mean serum calcium level in the pancreatectomized patients, 2.32 ± 0.03 mmol/L (9.3 ± 0.1 mg/dL), was at the lower limits of normal for our laboratory, undoubtedly reflecting the prevailing lowered serum albumin level.

In 10 men and three women, bone densitometry values were obtained during the postpancreatectomy follow-up period; this measurement was repeated at variable intervals in eight patients. The mean radial bone mineral content determinations in pancreatectomized men and women, expressed as a percentage of that obtained in age- and sex-matched normal subjects and depicted according to when the measurement was obtained after the surgical resection, are shown in Figure 1. While the bone density obtained in patients within 2 years after surgery appeared well maintained, patients who were studied more than 5 years after pancreatectomy had a mean reduction in bone mineral content of 17.6% compared to controls.

Pancreatogenic Diabetes

Insulin secretory reserve. Eighteen patients have had stimulated C-peptide levels measured after pancreatic resection, and in every instance the levels were undetectable (less than 0.02 nmol/L). This finding confirmed the completeness of the surgical procedure.

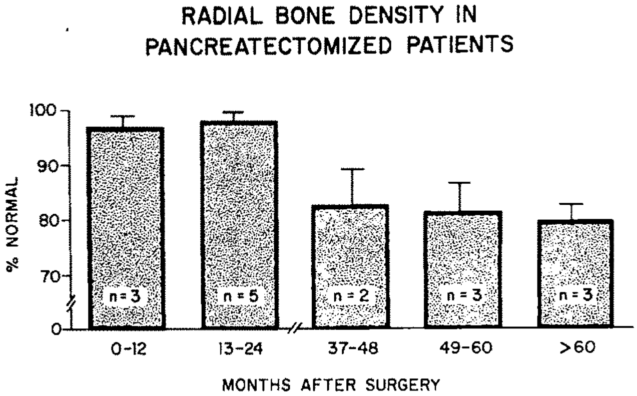

FIG. 1. Radial bone density in 10 pancreatectomized patients, expressed as a percentage of mean values obtained in age- and sex-matched normal subjects and plotted as a function of time (months) following pancreatic surgery. Total numbers of patients used to derive these mean values are indicated. Six patients had multiple values done, and they were meaned to give only a single value/patient for each respective time period depicted. Excluded were two patients with islet cell carcinomas and calcitonin production, and a patient with lymphoma who had evidence of osseous involvement.

Glucagon immunoreactivity and chromatographic profiles in pancreatectomized humans. Verified by chromatographic profiling in every instance, 3500-M_r glucagon was virtually absent in 18 pancreatectomized patients: for the group as a whole, 3500-M_r IRG comprised 1% to 2% of the total recovered IRG (Fig. 2). This pancreatic plasma IRG fraction was not stimulated by oral protein or intravenous arginine.

Glycemic lability. A pilot program of intensive home glucose monitoring was conducted to (1) assess the degree of diabetic instability existing in this population, (2) evaluate their insulin requirements, and (3) determine if near-

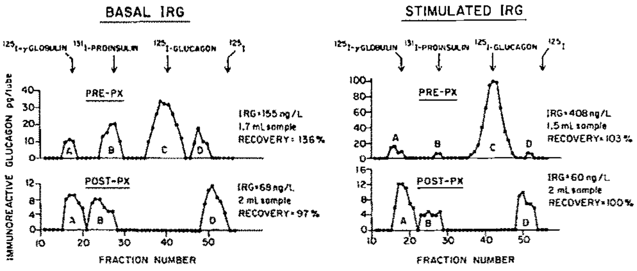

FIG. 2. Representative elution profiles of plasma IRG on 1 × 50 cm BioGel P-30 columns. Left panel shows the basal profile in a prepancreatectomy (top) or a postpancreatectomy (bottom) patient. Right panel shows the IRG response in a patient prepancreatectomy after intravenous arginine (top) *versus* that seen after pancreatectomy in response to oral protein (bottom). Arrows represent calibration points on column with labeled or unlabeled markers indicated.

euglycemia could be maintained without exacerbated hypoglycemia in the face of pancreatic glucagon deficiency.

Seven patients, free of recurrent disease at the time of evaluation, were asked to perform a fingerstick glucose measurement 1 hour before and after each meal (average, 8/day) for a 12- to 18-week period. The following mean determinations, obtained during the first month and repeated at the end of the study period, were used to assess the degree of diabetic instability: (1) fasting blood glucose; (2) the daily mean of the seven 1-hour postprandial glucose measurements; (3) blood glucose range, the maximal minus the minimal daily glucose concentration, modified from Reynolds et al.[30]; and (4) maximal excursion of blood glucose, the largest unidirectional consecutive change in blood glucose observed during the day.[31] For each of the four variables, the standard deviations for the 30 daily measurements in each of the initial and final periods were calculated to estimate day-to-day variation.

The initial mean fasting blood glucose level was 8.0 ± 1.1 mmol/L (142 ± 20 mg/dL), with a mean blood glucose level of 10.9 ± 1.4 mmol/L (200 ± 20 mg/dL). The variables describing plasma glucose fluctuation within a 24-hour period were compatible with values seen in patients with brittle diabetes[31]; the mean blood glucose range was 10.9 ± 1.4 mmol/L (194 ± 25 mg/dL), and the mean maximal excursion of blood glucose was 9.7 ± 1.2 mmol/L (173 ± 21 mg/dL). Fingerstick blood glucose determinations $\leq$2.8 mmol/L (50 mg/dL), often asymptomatic, occurred in the first month with a frequency of 0.9% to 8.6% in six patients; only the one patient with the highest glycemic indices was spared hypoglycemic episodes.

Figure 3 depicts the overall improvement in blood glucose control achieved with home glucose monitoring, as noted in serial glycosylated hemoglobin measurements. Hb A_1 levels on entry into the study were elevated in all patients (mean 11.3% ± 0.1%), and following intensive diabetic management, reached a nadir in most patients by 10 to 12 weeks, with five patients normalizing their glycosylated hemoglobin level during this period of observation. However, despite the continuation of the program for another 4 weeks, Hb A_1 levels started to increase after 12 weeks. Nevertheless an overall improvement in glycemic control persisted, with a significantly improved ($p < 0.01$) mean Hb A_1 of 9.4% ± 0.7% (normal range, 5.5% to 8.5%) at the end of the study documenting very reasonable diabetic control.

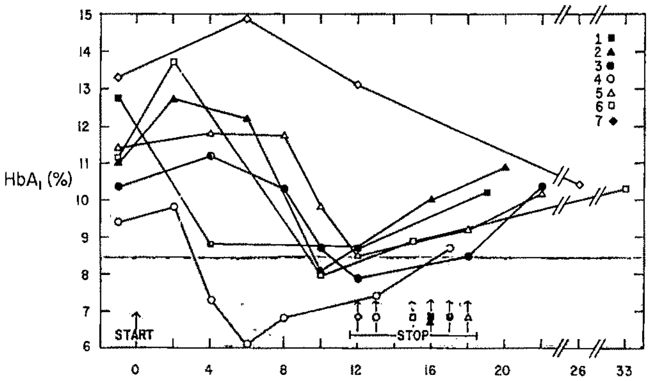

FIG. 3. Serial Hb A_1 (%) values plotted *versus* time (weeks). Solid arrows denote the times of starting and stopping the program of home glucose monitoring for all seven patients (depicted by symbols noted in the upper right hand corner). Stippled area represents our normal values for Hb A_1 (5.5%–8.5%).

In five patients significant improvement in parameters of diabetic control could be demonstrated after 3 months of intensive home glucose monitoring, and significantly decreased standard deviations for blood glucose and blood glucose range parameters suggested that these measurements became more stable in the final period with respect to day-to-day variation in every patient. Most importantly four patients experienced significantly less hypoglycemia.

Insulin requirements. The mean 24-hour insulin dose was 0.3[illegible] ± 0.03 U/kg body weight at time of discharge following pancreatectomy. In the long-term survivors, insulin requirements increased over the follow-up period of 74 to 174 months to 0.48 ± 0.04 U/kg body weight, probably as a result of their improved dietary intake. A mean total glycosylated hemoglobin (Hb A_1) level of 10.7% ± 0.3%, and, more recently, the mean Hb A_{1c} level of 8.5% ± 0.4% (normal ranges, 5.5% to 8.5% and 3.6% to 6.1%, respectively) confirmed that while normoglycemia was not achieved in these patients, reasonable diabetic control was indeed possible.

Long-term Sequelae

Patient performance status. Patients were able to resume reasonably active life styles within months of the surgery. Although every patient experienced a modification of their preillness regimen because of the necessity for frequent meals and blood glucose monitoring, most long-term survivors were able to return to their previous professional activities. No patient has remained home-bound or restricted in normal activity; vigorous physical exercise, however, is not recommended because of fear of hypoglycemia.

Diabetic sequelae. One patient died of documented hypoglycemia, which occurred shortly following hospital discharge after the pancreatic resection; in no other patients did symptomatic hypoglycemia occur that could not be treated at home. No patient required hospitalization for uncontrolled diabetes or diabetic ketoacidosis. No patient had evidence of diabetic retinopathy, as assessed by routine fundus examinations. Renal function was well maintained in all patients but two: one in whom renal insufficiency developed as a terminal event, probably related to underlying cardiac and septic complications, and another with concurrent Sjogren's syndrome. No patient had laboratory evidence of proteinuria. Nerve conduction studies were not performed, but symptomatic neuropathy developed in only one patient. Electrolyte and magnesium deficiencies secondary to severe chronic diarrhea may have been contributory.

Hepatic dysfunction. Three patients died of cirrhosis of the liver. In one, antecedent (prepancreatectomy) alcohol intake may have contributed. The second patient had moderate centrolobular steatosis documented at biopsy 8 years after surgery, which progressed within 1 year to fulminant end-stage liver disease and death. At autopsy the liver had fully developed irregular cirrhosis with moderate fatty changes throughout the nodule. Because of clinically significant liver disease, the third patient had a liver biopsy 3 years after pancreatectomy and focal (minimal) steatosis was documented. The patient died of complications of hepatic insufficiency 3 years later. At autopsy fully developed irregular cirrhosis was noted, with moderate to severe steatosis throughout the nodule. Three other long-term survivors (7, 9, and 10 years after pancreatectomy) have biopsy and/or computed tomography (CT) scan evidence of fatty infiltration of the liver.

Other malignancies. Two patients with adenocarcinoma of the pancreas had a second malignancy (ovarian cancer found incidentally at time of pancreatectomy and breast cancer noted 6 months after pancreatic resection). Two other patients (one with chronic pancreatitis, the other with periampullary carcinoma) developed colon cancer 51 and 47 months, respectively, after pancreatectomy.

Discussion

Significant clinical morbidity can be expected to result from the physiologic sequelae of the surgical procedure, which includes gastric antral resection, cholecystectomy with resection of the common bile duct, pancreatectomy, and interruption in intestinal innervation and lymphatic drainage. The total pancreatectomy and intestinal denervation appear to cause the most severe physiologic abnormalities, resulting in acutely disordered intestinal function and a chronic diabetic state uniquely characterized by a lack of the major glucoregulatory hormones insulin and glucagon.

The early postoperative state is marked by diarrhea, which hampers timely convalescence and hospital discharge. Multiple aspects of the surgical procedure can result in impaired gastrointestinal motility, absorption, and secretion. Pancreatic exocrine deficiency *per se*, with maldigestion, malabsorption, and steatorrhea, results in excessive fecal fluid losses.[32] Faster cycling of the bile salt pool reported after cholecystectomy may lower the diarrheal threshold as well.[33] We could not note a difference in the postoperative course of the two patients who did not have regional total pancreatectomy. However the diarrhea experienced by patients in the current series does seem to be of greater magnitude than that reported after standard total pancreatic resection[6,9] or after pyloric- and gastric-preserving pancreatic resection.[34]

Unique to the regional total pancreatectomy are the lymphatic interruption and surgical sympathectomy. Lacking information on mucosal morphology in this population, the exact role of altered lymphatic drainage is unclear. Intestinal denervation, however, can result in diarrhea by at least two distinct mechanisms. Resection of the celiac and superior mesenteric ganglia affects small intestinal motility *via* loss of the tonic inhibitory effects of sympathetic nerves on gastrointestinal function.[35] In addition the altered homeostatic balance of intestinal ion transport favors secretion.[36] Remarkably the diarrhea abates spontaneously in most patients after a variable period of time. However, in the acute postoperative state, bile acid sequestrants, somatostatin analogues, and alpha-2-adrenergic agonists may be found beneficial in future controlled studies.

Previous investigators have found weight loss in 30% to 40% of patients and weight maintenance to be a significant chronic problem after total pancreatectomy.[7,8] In addition deficiencies of fat-soluble vitamins[37] and trace elements[38] are common in patients with chronic exocrine pancreatic insufficiency despite adequate pancreatic supplements. The current experience differs from that expected in this patient population. After the early postoperative period, patients free of recurrent disease gained or maintained weight in every instance despite the presence of modest steatorrhea. Furthermore nutritional deficiencies were prevented in all but a small number of patients. This was accomplished by conventional enzyme replacement therapy (at least 20,000 U lipase/major meal), routine calcium and vitamin supplementation, and scrupulous adherence to a regimen of multiple calorie-dense meals per day without dietary fat restriction. A high caloric requirement was a consistent finding in all patients, not fully explained by the degree of steatorrhea present. Basal metabolic rate determinations have not been performed and may be of interest in such patients. Because of the persistence of severe hypoalbuminemia, one patient underwent a detailed investigation for a protein-losing enteropathy, with negative results.

Given the antral resection and malabsorption, it is expected that the pancreatectomized patient will be at risk for the development of osteopenia,[39,40] and these data, while clearly preliminary, support this. Despite measures that prevented calcium and vitamin D deficiencies in most patients, radial bone mineral content was decreased with time after surgery. The longitudinal use of the more sensitive technologies currently available, such as vertebral/femoral neck dual photon or dual-energy x-ray absorptiometry or CT scanning,[41,42] is recommended, so that more aggressive, bone-preserving strategies could be instituted promptly when indicated.

The diabetes of the totally pancreatectomized patient has unique clinical and metabolic features. Diabetic instability is a constant finding. There is an erratic pattern of glycemic fluctuations, an impressive postprandial hyperglycemia, and delayed, often nocturnal decreases in blood glucose levels. The 'brittle' nature of blood glucose profiles is similar to that seen in young patients with insulin-dependent diabetes mellitus (IDDM) and is not improved by glucagon replacement.[43] Thus it would appear that endogenous insulin reserve is a primary determinant of diabetic stability in patients with pancreatogenic diabetes, as has been suggested in other diabetic populations.[30,44,45]

An important characteristic of the labile diabetic milieu in this population is the ease with which hypoglycemia can be induced. Review of the surgical literature confirms the increased susceptibility of these patients to severe hypoglycemia, with brain dysfunction, coma, and death as a sequelae of insulin therapy reported in several large surgical series.[6-8,46] The incidence of hypoglycemia, often asymptomatic, was unacceptably high in this series until the institution of rigorous home glucose monitoring and administration of multiple, small daily doses of insulin as required. It was not possible to achieve persistent normalization of glycemic control in any patient in this study. However, given the morbidity of uncontrolled glycemic excursions, vigorous efforts at blood glucose stabilization in a population of pancreatectomized patients are essential.

This labile and 'brittle' pattern of glycemic control has led to the widespread clinical impression that patients with pancreatogenic diabetes are unusually insulin sensitive. Previous uncontrolled studies have suggested that totally pancreatectomized patients need less insulin than patients with IDDM.[47,43] This has been confirmed by a study using Biostator glucose-controlled insulin infusion system (Life Science Instruments, Ulm, Germany) and identical inpatient dietary regimens: following total pancreatectomy, a significant and substantial ($\approx$35%) decrease in insulin requirements was noted compared to patients with subtotal pancreatectomy or IDDM.[49]

The locus of enhanced insulin responsiveness does not appear to be peripheral tissues. While enhanced peripheral glucose uptake in response to insulin was reported in patients with pancreatogenic diabetes,[50] more recent studies in well-characterized completely pancreatectomized patients have demonstrated significant insulin resistance in this population, comparable to that seen in patients with IDDM.[51,52] Indeed basal glucagon replacement induced a further significant reduction in tissue insulin responsiveness.[53]

The primacy of glucagon in the maintenance of postabsorptive glucose concentrations is well established. Where the completeness of the surgical procedure was established, studies using column chromatography have detected absent or markedly reduced 3500-M_r glucagon in totally pancreatectomized patients.[28,54] Impaired counter-regulatory hormone responsiveness has been demonstrated in these patients; two separate laboratories have found no increase of glucagon and a reduced[55] or delayed[56] epinephrine response to insulin-induced hypoglycemia. Certain metabolic features in this diabetic state are consistent with a chronic lack of biologically active pancreatic glucagon as well: the delayed increase of blood glucose and ketone bodies after cessation of insulin therapy,[57] the absence of an increase in blood glucose during arginine infusion,[58,59] and the abnormally elevated concentrations of the major glucogenic amino acids that decrease with physiologic glucagon replacement.[43,49,60,61] Furthermore pancreatectomized patients have an exaggerated hyperglycemic response to glucagon administration compared to that seen in comparably insulin-deficient patients with IDDM, suggesting that chronic glucagon deficiency enhances sensitivity to exogenous glucagon.[43] It would appear, therefore, that impaired regulation of hepatic glucose production subsequent to surgically induced pancreatic alpha cell insufficiency is a probable cause for the frequent hypoglycemia seen in these patients. Whether glucagon replacement therapy in this population would reduce the incidence of this morbid complication remains an intriguing therapeutic option worthy of further study.

An uncommon but worrisome finding in prolonged follow-up of totally pancreatectomized patients has been the development of accelerated lipid accumulation in the liver, with end-stage liver disease as a cause of death noted in three such patients in this study. This complication has been described as well in experimental pancreatectomized animals.[62,63] While poorly controlled diabetes, weight loss, and malabsorption can be contributing factors, the severity of hepatic steatosis noted in patients who have been in reasonable diabetic control suggests additional pathogenic mechanisms. Preliminary information suggests that the storage triglycerides are not dietary in origin but result from altered hepatic metabolism. The impact of the altered hormonal milieu of the postpancreatectomy state on hepatic lipogenesis is now being studied in our laboratory.

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The Complex Pattern of Cytokines in Sepsis

Association Between Prostaglandins, Cachectin, and Interleukins

WOLFGANG ERTEL, M.D.,* MARY H. MORRISON, M.S.,* PING WANG, M.D.,* ZHENG F. BA, B.S.,*
ALFRED AYALA, PH.D.,*† and IRSHAD H. CHAUDRY, PH.D.*‡

Although the cytokines tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6) are important mediators of hemodynamic, metabolic, and immunologic alterations in the host during sepsis, it is not known whether there is any association between the release of these cytokines and prostanoids during sepsis. Sepsis induced by cecal ligation and puncture in rats led to a persistent elevation ($p < 0.05$) of plasma TNF until 10 hours, steadily increasing ($p < 0.05$) IL-1 plasma levels, and enhanced ($p < 0.05$) IL-6 plasma levels at all time points compared to the sham group. Prostaglandin E_2 plasma levels were elevated ($p < 0.05$) at 5 hours (153 ± 29 pg/mL; control: 47 ± 11 pg/mL) and 10 hours (96 ± 16 pg/mL; control: 21 ± 5 pg/mL). Prostaglandin E_2 production by splenic macrophages (sMϕ) from septic animals was increased ($p < 0.05$) at 5 hours (9.1 ± 2.2 ng/mL) and 10 hours (5.6 ± 1.5 ng/mL) compared to controls (3.3 ± 0.3 ng/mL at 5 hours; 1.3 ± 1.3 ng/mL at 10 hours). Incubation of sMϕ from septic animals with ibuprofen enhanced ($p < 0.05$) IL-1 and TNF synthesis, while IL-6 production was reduced ($p < 0.05$). These results indicate that the alterations in prostanoid release and elevated plasma prostanoids may regulate the release and consequently the circulating levels of cytokines during sepsis.

From the Departments of Surgery, Microbiology and Public Health,† and Physiology,‡ Michigan State University, East Lansing, Michigan*

THE RELEASE OF cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and cachectin (tumor necrosis factor [TNF]) by macrophages is significantly altered during endotoxemia, sepsis, and septic shock.[1-6] Tumor necrosis factor, in particular, appears to play a pivotal role in lethal endotoxemia.[7-8] Anti-TNF antibodies attenuated the lethal effect of endotoxemia and prevented the development of lethal septic shock syndrome.[9-11] Tumor necrosis factor induces the synthesis of monokines, such as IL-1β[12] and IL-6,[13] which have been implicated in the pathophysiology of infection.[14,15] Passive immunization against TNF attenuated the appearance of IL-1β and IL-6,[16] indicating that TNF is the initial stimulus for the release of these cytokines during the septic syndrome. Furthermore administration of recombinant IL-1 or IL-6 induced some of the characteristic physiologic derangements associated with injury,[17,18] including fever and acute-phase protein responses.

In addition to enhanced cytokine release, macrophages produce large amounts of arachidonic acid metabolites in response to endotoxin, IL-1, and TNF.[19-21] Prostaglandins are potent immunosuppressive mediators.[22] *In vitro* studies[23-25] using macrophages from unmanipulated animals have shown that prostaglandins, in particular prostaglandin E_2 (PGE_2), downregulate their inducer cytokines, IL-1 and TNF, through a negative feedback mechanism. The effect of prostaglandins on the synthesis and release of IL-6 during sepsis, however, is not known. Although the above-referenced *in vitro* studies clearly indicate a regulatory role of prostaglandins on cytokine release, the kinetics of circulating PGE_2 in the early and the late stages of sepsis, its synthesis by macrophages, and its role in altering the release of cytokines remain unknown.

It was, therefore, the aim of this study to correlate the alterations in plasma TNF, IL-1, and IL-6 levels with plasma PGE_2 levels in sepsis. Furthermore PGE_2 synthesis by splenic macrophages was measured and the effect of cyclooxygenase blockade by ibuprofen on splenic macrophage cytokine release was determined.

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Dr. Ertel's present address is Chirurgische Klinik und Poliklinik der, Ludwig-Maximilians-Universitaet, Muenchen, Klinikum Grosshadern, Marchioninistrasse 15, D-8000 Munich 70, Germany.

Address reprint requests to Irshad H. Chaudry, Ph.D., Department of Surgery, B424 Clinical Center, Michigan State University, East Lansing, MI 48824-1315.

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Materials and Methods

Experimental Protocol

The experimental protocol and the care of the animals were carried out according to the guidelines set forth by the Animal Welfare Act and with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publications.

Male Sprague-Dawley rats, weighing 260 to 360 g, were fasted overnight. After administration of light ether anesthesia, sepsis was induced by cecal ligation and puncture (CLP), as described in detail previously.[26] Briefly, after a 2-cm midline incision was made, the cecum was isolated and a 3-0 silk ligature was placed around it, ligating the cecum just below the ileocecal valve (without interrupting the continuation of the small bowel and the colon). The cecum was then punctured twice with an 18-gauge needle, replaced in the peritoneal cavity, and the abdominal incision closed in two layers. Saline (3 mL/100 g body weight) was given subcutaneously at this time. Laparotomy without CLP was performed in control (sham) animals. Animals from both groups were killed at different time points (2, 5, 10, and 20 hours) after CLP or sham laparotomy. Plasma for cytokine determinations was collected and the spleen harvested. To obtain cytokine and endotoxin baseline values in plasma, blood was collected from unmanipulated rats by cardiac stick.

Preparation of Splenic Macrophages

Spleens were harvested, gently ground between frosted slides, and resuspended in Click's medium (Irvine Scientific, Santa Ana, CA). Erythrocytes were lysed by hypotonic shock. The remaining cells were washed twice and resuspended in Click's medium. Cell viability, tested by Trypan Blue exclusion, was ≥95% and did not reveal any differences between the septic and the sham-operated group. After determination of splenic macrophage (sMϕ) numbers present in the cultures, 1×10^6 sMϕ/mL Click's medium was transferred to 24-well plates and incubated for 4 hours (at 37 C, 5% CO_2). Nonadherent and nonviable cells were removed by five repeated washings of the well. This protocol provided cultures that were more than 95% Mϕs, as determined by nonspecific esterase staining, and exhibited typical Mϕ morphology. The remaining adherent sMϕ were incubated further for 24 hours in Click's medium containing 10% fetal bovine serum (Biologos Inc., Naperville, IL) with 10 μg/mL (for cytokine measurement) or 1 μg/mL (for PGE_2 measurement) lipopolysaccharide W (LPS, from *Escherichia coli* 055:B5, Difco Labs., Detroit, MI). For studying the *in vitro* effects of prostaglandins on cytokine production, the cyclooxygenase inhibitor ibuprofen (10 μg/mL, Sigma Chemical Co., St. Louis, MO) was added to sMϕ cultures. Supernatants of sMϕ were harvested, filtered, aliquoted, and frozen at −70 C until assayed.

Interleukin-1 Assay

The activity of IL-1 in plasma samples and sMϕ supernatants was determined by incubating serial dilutions of samples with D10.G4.1 cells (2×10^4 cells/well)[27] in the presence of Concanavalin A (Pharmacia Fine Chemicals, Piscataway, NJ). Recent studies[28] have shown that D10.G4.1 cells do not react with recombinant IL-6. This observation is important for the determination of IL-1 in plasma because following endotoxemia, significant amounts of IL-6 are present in plasma.[16] However we cannot preclude the possibility that there might be interference with other cytokines, such as IL-2, IL-4, or IL-7, in this assay.[28] After an incubation period of 48 hours, D10.G4.1 proliferation was measured by incorporation of ^{3}H-thymidine. The unit(s) of IL-1 activity was calculated, comparing the slope of the sample curve to that of the standard curve according to the method of Mizel.[29] One unit was defined as the amount of the cytokine required to produce one-half maximal proliferation.

Interleukin-6 Assay

The activity of IL-6 was measured by the proliferation of the 7TD1 hybridoma.[30] Briefly, serial dilutions of plasma or supernatant were incubated in duplicate with 4×10^4 7TD1 cells/mL for 72 hours in 96-well plates and then MTT tetrazolium (Sigma Chemical Co.) was added. Four hours later the supernatants were removed and the cells lysed with 0.04 N HCl in isopropanol. The degree of cell proliferation was determined colorimetrically in an enzyme-linked immunoadsorbent assay plate reader (620 nm). The units of activity in the samples were calculated as described above.[29]

Tumor Necrosis Factor Assay

Tumor necrosis factor activity was determined by its cytotoxic effect on the fibrosarcoma cell line WEHI 164 subclone 13.[31] The degree of lysis induced by TNF was measured by proliferation of nonlysed WEHI cells using the MTT assay as described above.

It should be noted that the IL-6 and TNF assay have been shown to react in a highly specific fashion to TNF or IL-6, respectively.[32,33]

PGE_2 Radioimmunoassay

Prostaglandin E_2 levels in plasma and sMϕ supernatants were measured using a radioimmunoassay technique (NEN, DuPont, Boston, MA; cross-reactivity with PGE_1: 3.7%, $DHKPGE_2$:0.4%, PGA_2:0.04%, other prostaglandins and arachidonic acid: <0.01%) according to the

TABLE 1. *Circulating Endotoxin Levels Following Cecal Ligation and Puncture Compared to Sham Laparotomized Animals*

Group	(Endotoxin U/mL Plasma) Time After CLP 2 Hours	5 Hours	10 Hours	20 Hours
Sham (n = 4)	0.64 ± 0.56	0.53 ± 0.41	0.86 ± 0.90	1.30 ± 0.19
CLP (n = 4)	4.33 ± 1.45*	3.41 ± 2.03*	5.51 ± 3.25	9.51 ± 4.55*

Baseline values of endotoxin in plasma of unmanipulated animals: 0.07 ± 0.01 EU/mL.

* $p < 0.05$ compared to sham (Wilcoxon rank sum test).

manufacturer's guidelines. Control studies revealed a 25% to 30% contamination of the plasma with arachidonic acid. Therefore PGE_2 was extracted and purified from plasma using absorption and elution from Bond-Elut C_{18} and SI columns (Analytichem International, Harbor City, CA) before testing for PGE_2, as described previously.[34]

Endotoxin Assay

Rat plasma was diluted 1:100 in pyrogen free water and heated at 100 C for 10 minutes to remove nonspecific inhibitors before measuring endotoxin levels using the quantitative chromogenic Limulus Amebocyte Lysate assay kit (QCL-1000; Whittaker, M.A. Bioproducts, Walkersville, MD). All samples were tested in duplicate.

Statistical Analysis

All data are expressed as mean ± standard error of the mean. Four experiments per group at each time point were performed. Comparison between the means of the two experimental groups was carried out separately for each time point using the unpaired t test or Wilcoxon rank sum test (used for endotoxin levels). One-way analysis of variance (ANOVA) was used to compare baseline values of unmanipulated animals to values of sham-operated and septic animals. The paired t test was applied to compare the effect of ibuprofen on cytokine production in sMϕ cultures. Values were considered significant if p was less than 0.05.

Results

Endotoxin Plasma Levels

Measurement of endotoxin levels in plasma revealed a marked increase of endotoxin in septic animals at all four time points, with a significant elevation ($p < 0.05$) at 2, 5, and 20 hours compared to sham-operated animals (Table 1). Endotoxin plasma levels in septic animals were increased steadily with peak levels at 20 hours (9.51 ± 4.55 EU/mL; sham: 1.30 ± 0.19 EU/mL).

Cytokine Plasma Levels

Circulating TNF (Fig. 1A) in septic animals was significantly ($p < 0.05$) increased in the first 10 hours when compared to sham-operated animals. The peak levels in the septic group were detected at 2 hours (4.24 ± 0.55 U/mL) with decreasing levels toward 20 hours (2.53 ± 1.99 U/mL). Unmanipulated control animals did not reveal detectable TNF levels in plasma. Circulating IL-1 in septic animals was elevated during the entire observation period, with significantly higher levels ($p < 0.05$) at 5, 10, and 20 hours compared to plasma IL-1 levels of sham-operated animals (Fig. 1B). Peak levels of IL-1 in the septic group were observed at 20 hours (15.53 ± 3.80 U/mL; sham: 0.42 ± 0.14 U/mL). Both groups had significantly ($p = 0.0001$ [sham]; $p = 0.00004$ [CLP]; one-way ANOVA) higher IL-1 plasma levels than unmanipulated animals (0.04 ± 0.02 U/mL). Circulating IL-6 levels were significantly ($p < 0.05$) elevated in the septic group during the entire observation period, with peak levels at 10 hours (62.1 ± 18.1 U/mL) compared to sham-operated animals (5.4 ± 1.1 U/mL) (Fig. 2A). Both groups, sham-operated and CLP animals, showed significantly ($p = 0.03$ [sham]; $p = 0.0067$ [CLP]) higher IL-6 plasma levels than unmanipulated animals (0.83 ± 0.26 U/mL).

PGE_2 Plasma Levels

Plasma levels of PGE_2 in septic animals were significantly increased at 5 hours (153.5 ± 28.6 pg/mL) and 10 hours (95.8 ± 16.2 pg/mL) ($p < 0.05$ at 5 hours; $p < 0.01$ at 10 hours) compared to sham-operated animals (46.5 ± 10.8 pg/mL at 5 hours; 20.8 ± 5.2 pg/mL at 10 hours) (Fig. 2B). Peak levels of PGE_2 in the septic group were observed at 5 hours. At 2 hours and 20 hours, circulating PGE_2 in the septic group was not increased compared to sham-operated animals. Septic animals had significantly ($p = 0.0002$) higher PGE_2 plasma levels in comparison to unmanipulated animals (43.0 ± 10.1 pg/mL), while circulating PGE_2 levels of sham animals were not different ($p = 0.1$) from unmanipulated animals.

PGE_2 Release by Splenic Macrophages

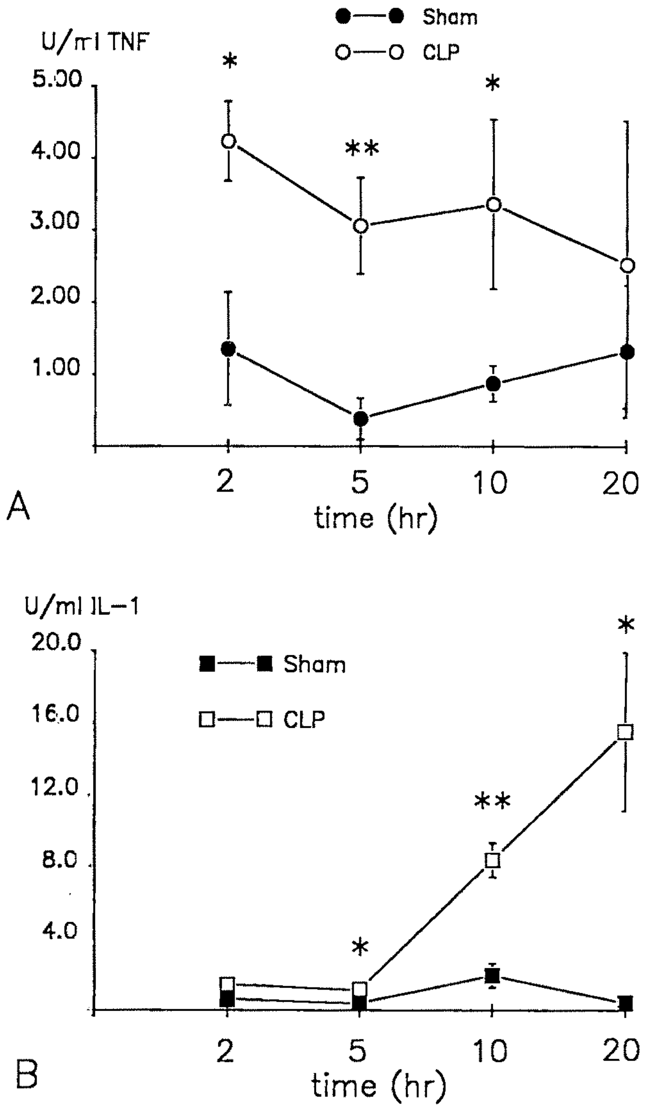
The capacity of splenic macrophages from septic animals to release PGE_2 was increased at 5 hours and 10 hours after the onset of sepsis (Fig. 3). Prostaglandin E_2 release by splenic macrophages from septic animals was three times higher (9.08 ± 2.23 ng/mL; $p < 0.05$) at 5 hours and four times higher (5.56 ± 1.48 ng/mL, $p < 0.01$) at 10 hours than in the sham-operated group (3.30 ± 0.34 ng/mL at 5 hours; 1.33 ± 1.30 ng/mL at 10 hours) (Fig. 3). At 20 hours there was still a 143% increase in PGE_2 release by Mϕ in the septic group (7.44 ± 4.83 ng/mL) compared to the sham group (3.06 ± 1.19 ng/mL), which was not found to be statistically different.

Effect of Cyclooxygenase Inhibition on Mϕ Cytokine Release

Incubation of splenic macrophage cultures with 10 μg/mL ibuprofen in the presence of LPS resulted in a complete inhibition of PGE_2 production because no detectable amounts of PGE_2 in these supernatants were observed. Ibuprofen increased splenic macrophage TNF release from septic animals by 95% at 5 hours (3.04 ± 0.51 U/mL) and by 79% at 10 hours (2.38 ± 0.26 U/mL; $p < 0.05$) when compared to untreated cultures (1.56 ± 0.42 U/mL at 5 hours and 1.33 ± 0.23 U/mL at 10 hours) (Fig. 4). Interleukin-1 release by splenic macrophages from septic animals was significantly ($p < 0.01$) enhanced by 413% at 10 hours with ibuprofen treatment (2.12 ± 0.49 U/mL) compared to untreated splenic macrophage cultures (0.41 ± 0.22 U/mL) (Fig. 5). In contrast to IL-1 and TNF, IL-6 synthesis by splenic macrophages from septic animals was either unaltered or decreased (5 hours, $p < 0.05$) after incubation with ibuprofen (Fig. 6). The addition of ibuprofen to sMϕ cultures from sham-operated animals did not produce any significant effect on the release of TNF, IL-1, or IL-6 (Figs. 4 to 6). Furthermore IL-6 release by splenic macrophages from septic animals (36.1 ± 12.0 U/mL) was markedly ($p < 0.05$) increased at 10 hours when compared to sham-operated animals (6.3 ± 1.4 U/mL).

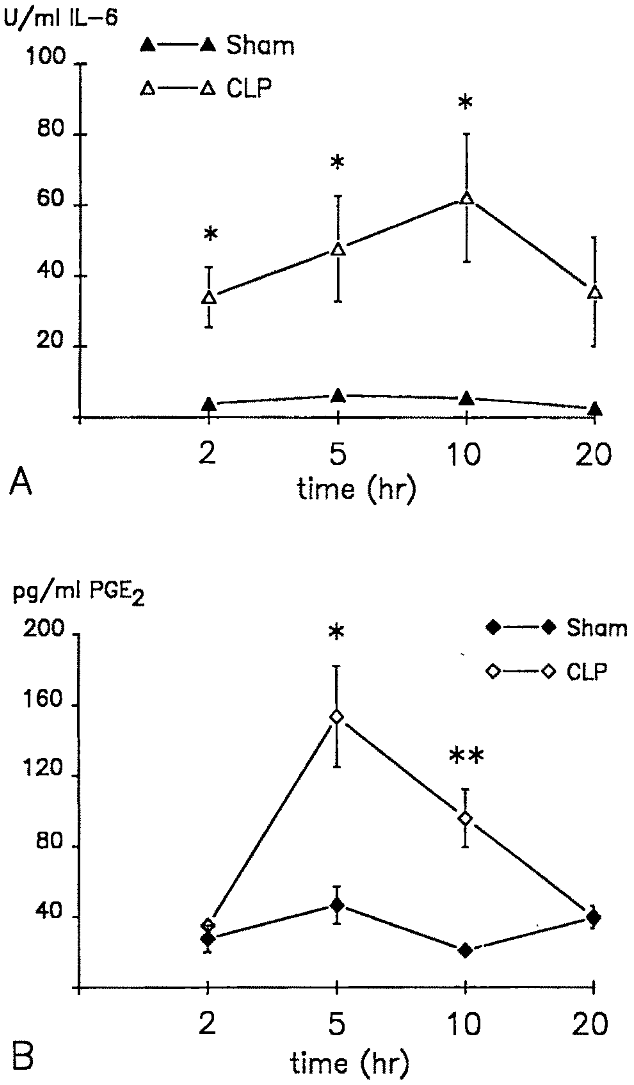
Discussion

Sepsis and septic shock remain a serious problem in intensive care units and patients in sepsis often develop physiologic and inflammatory derangements that lead to a common syndrome of multiple-organ failure and death. Although the precise etiology of these often-lethal complications caused by sepsis may be multifactorial, a frequent common denominator of organ damage and consequently organ failure are alterations in cytokine release, predominantly by macrophages. Serum profiles of TNF, IL-1, and IL-6 and their interactions have been well described following endotoxin administration in both animal experiments[2,16] and patient studies.[1,35] The results demonstrated an early, but transient, peak in TNF followed by an increase in IL-1β and IL-6. Using monoclonal antibodies against TNF, Fong et al.[16] showed that TNF is a potent inducer of IL-1β and IL-6 release. Shalaby et al.[2] further suggested that besides endotoxin and TNF, IL-1 is able to upregulate IL-6. These above-referenced studies were carried out using a bolus injection of endotoxin. It is possible, however, that the alterations of cytokine release induced by a persistent septic focus may differ from the changes observed following a bolus injection of endotoxin.



FIGS. 1A and 1B. Circulating TNF (A) and IL-1 (B) plasma levels 2, 5, 10, and 20 hours after CLP (O–O) or sham laparotomy (●–●) compared with values from unmanipulated controls (TNF: not detectable; IL-1: 0.04 ± 0.02 U/mL). TNF (U/mL) and IL-1 (U/mL) levels were measured using the specific cell lines WEHI 164 subclone 13 for TNF and D10.G4.1 for IL-1. Four experiments per group at each time point were carried out. The data are presented as mean ± SEM. *$p < 0.05$./**$p < 0.01$ CLP *versus* sham laparotomy.

Clinical studies[1,3,4-6,36,37] examining serum cytokine levels have demonstrated that TNF and IL-1 blood levels are significantly elevated in patients with sepsis. In addition Nijsten et al.[15] described an increase in IL-6 during infectious episodes in humans. Although these studies indicated some of the similarities between the results observed in septic patients and animal studies using endo-



FIGS. 2A and 2B. Circulating IL-6 (A) and PGE_2 (B) plasma levels 2, 5, 10, and 20 hours after CLP (O–O) or sham laparotomy (●–●) compared with values from unmanipulated controls (IL-6: 0.83 ± 0.26 U/mL; PGE_2: 43.0 ± 10.1 pg/mL). IL-6 (U/mL) was determined with a bioassay using the specific cell line 7TD1 and PGE_2 (pg/mL) was measured with RIA technique after extracting PGE_2 from plasma. Four experiments per group at each time point were carried out. The data are presented as mean ± SEM. $^*p < 0.05$/$^{**}p < 0.01$ CLP *versus* sham laparotomy.

toxin administration, the fact remains that most of these studies only determined one or two cytokines at the same time point. This, together with the lack of a narrow screening on consecutive time points, however, make it difficult to compare the kinetics and interactions of the different cytokines. Furthermore the kinetics of PGE_2, a potent immunomodulatory mediator[22] in plasma, and its production by macrophages during endotoxemia or sepsis are unknown. Because there is evidence from many *in vitro* studies[23-25] using cells from unmanipulated animals that PGE_2 regulates the synthesis and release of these cytokines, PGE_2 might play a pivotal role in the control of IL-1, IL-6, and TNF secretion by macrophages during sepsis.

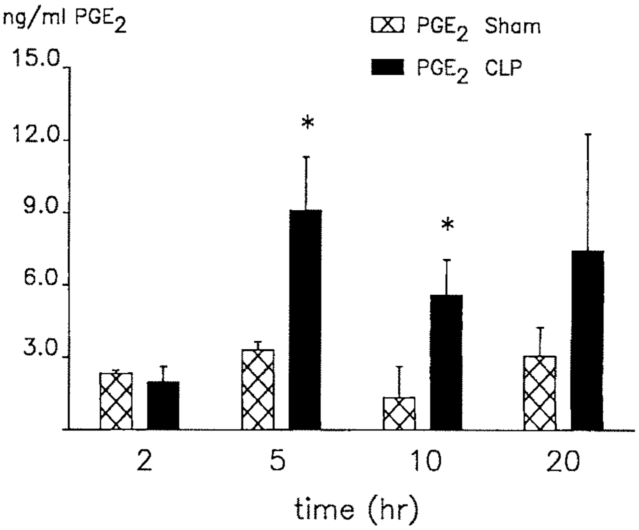


FIG. 3. Production of PGE_2 (ng/mL) by splenic macrophages from CLP animals and sham laparotomized animals at 2, 5, 10, and 20 hours after the insult. Splenic macrophages were stimulated with LPS (1.0 μg/mL) for 24 hours. PGE_2 was determined with radioimmunoassay. Four experiments per group at each time point were carried out. The data are presented as mean ± SEM. $^*p < 0.05$ CLP *versus* sham laparotomy.

Most of the previous studies used endotoxin administration to study the events in septic shock. It remains to be determined, however, whether cytokine production is altered in a clinically relevant model of sepsis, such as cecal ligation and puncture.[26] Endotoxin administration produces either the hyperdynamic or hypodynamic state

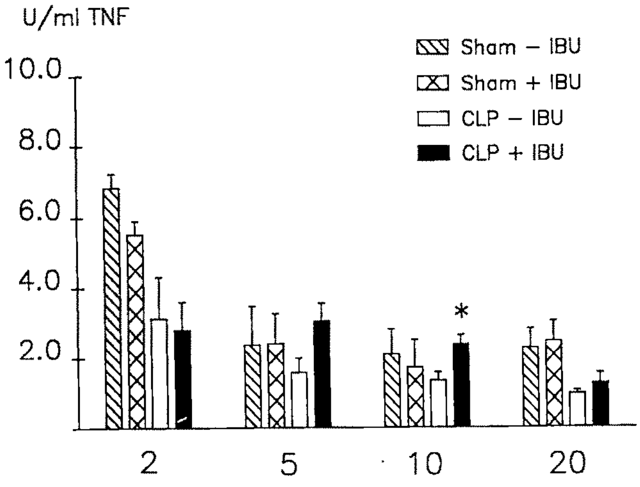


FIG. 4. Effect of ibuprofen on production of TNF by splenic macrophages. Splenic macrophages were obtained from animals 2, 5, 10, and 20 hours after sham operation or CLP, stimulated with LPS (10.0 μg/mL) and incubated with or without ibuprofen (10 μg/mL) for 24 hours. Release of TNF [U/mL] was measured in supernatants of sMϕ using the WEHI 164 subclone 13 cell line. Four experiments per group at each time point were carried out. The data are presented as mean ± SEM. $^*p < 0.05$ − *versus* + ibuprofen.

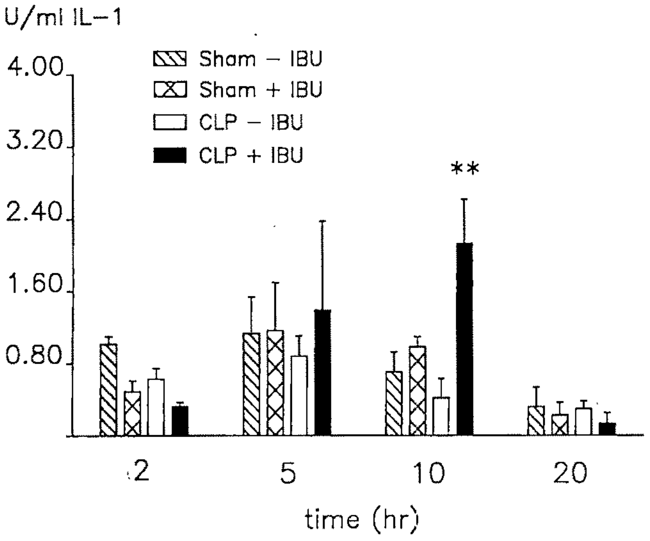


FIG. 5. Effect of ibuprofen on production of IL-1 by splenic macrophages. Splenic macrophages were obtained from animals 2, 5, 10, and 20 hours after sham operation or CLP, stimulated with LPS (10.0 μg/mL) and incubated with or without ibuprofen (10 μg/mL) for 24 hours. Release of IL-1 [U/mL] was measured in supernatants of sMϕ using the D10.G4.1 cell line. Four experiments per group at each time point were carried out. The data are presented as mean ± SEM. $^{**}p < 0.01$ – *versus* + ibuprofen.

dependent on the dosage used but lacks a consistent septic focus in combination with devitalized tissue. Cecal ligation and puncture, however, produces an early hyperdynamic, hypermetabolic state, followed by a hypodynamic, hypometabolic state and thus mimics the characteristics of sepsis seen in humans.[26]

The present data indicate that the levels of circulating TNF and IL-1 during sepsis differ from those observed after a bolus injection of endotoxin. Following injection of endotoxin, circulating TNF and IL-1 levels increased in a transient fashion during the first 3 hours but were not detectable after 4 hours.[1,2,16] However, during sepsis, TNF was elevated at 2, 5, and 10 hours following cecal ligation and puncture (CLP), while IL-1 levels initially increased at 5 hours and thereafter increased until the end of the observation period of 20 hours. This could be due to the presence of a persistent septic focus and steadily increasing plasma levels of endotoxin. However this does not explain the tendency of circulating TNF to decrease toward 20 hours.

In contrast to TNF and IL-1, the kinetics of circulating IL-6 following CLP was similar to that seen after a bolus injection of endotoxin. The steadily increasing levels of circulating IL-6 up to 10 hours after CLP are similar to those described by Fong et al.,[16] who observed increasing plasma levels of IL-6 for up to 8 hours after endotoxin administration. Our data further demonstrate that the immunomodulatory eicanosoid PGE_2 is significantly elevated in plasma during sepsis, exhibiting a transient peak at 5 hours. The delayed appearance of IL-6 and PGE_2 may indicate that, in addition to endotoxin, other mediators such as TNF and IL-1 are necessary costimulatory factors for initiating the production of PGE_2. This suggestion is in line with several *in vitro* and *in vivo* studies[38–42] that demonstrated a pivotal role of TNF and IL-1 in the stimulation of PGE_2 and IL-6 synthesis and release.

In contrast to the studies involving a bolus injection of endotoxin, which show a rise and then a rapid decrease in plasma TNF (perhaps due to the clearance of endotoxin from the circulation), circulating TNF following CLP was highest at 2 hours and tended to decrease at all time points thereafter, despite increasing plasma endotoxin levels. This suggests that TNF production and release may be downregulated by mediators other than endotoxin with the progression of sepsis. Because *in vitro* studies demonstrated that PGE_2 suppresses TNF mRNA accumulation and synthesis,[25,43] PGE_2 may be a mediator that can control TNF synthesis and release by macrophages in the late stage of sepsis. This hypothesis is supported by our *in vitro* studies, which showed that blockade of cyclooxygenase increased TNF release by splenic macrophages from septic animals but not from sham-operated animals. Furthermore steadily increasing plasma levels of IL-1 were observed in the late stages of sepsis. Our *in vitro* studies indicate that PGE_2 may be involved in the regulation of IL-1 release by macrophages because addition of ibuprofen

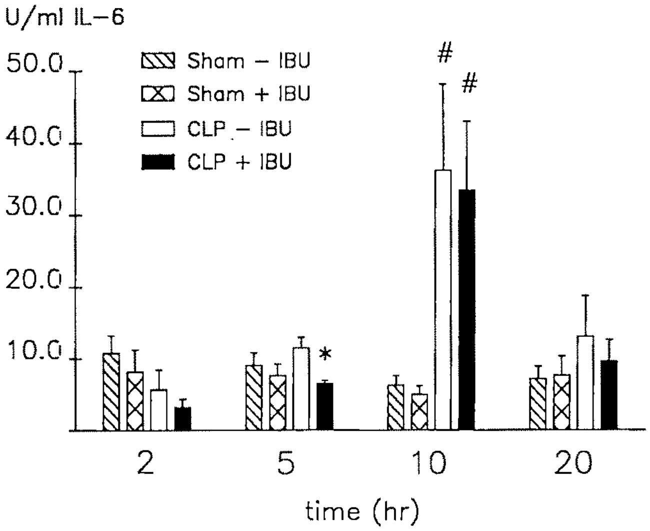


FIG. 6. Effect of ibuprofen on production of IL-6 by splenic macrophages. Splenic macrophages were obtained from animals at 2, 5, 10, and 20 hours after sham operation or CLP, stimulated with LPS (10.0 μg/mL) and incubated with or without ibuprofen (10 μg/mL) for 24 hours. Release of IL-6 [U/mL] was measured in supernatants of sMϕ using the 7TD1 cell line. Four experiments per group at each time point were carried out. The data are presented as mean ± SEM. $^{*}p < 0.05$ – *versus* + ibuprofen; $\#p < 0.05$ CLP *versus* sham.

to splenic macrophage cultures of septic animals increased IL-1 release. This was not observed in splenic macrophage cultures of sham-operated animals. The significance of these high IL-1 plasma levels remains unclear so far. However, because Okusawa et al.[44] described a shocklike state in rabbits after infusion of high doses of IL-1, and Waage et al.[45] reported that IL-1 can potentiate the lethal effects of TNF, we propose that the observed elevated plasma IL-1 levels may be, at least in part, responsible for the shocklike state in our animals in the late stages of sepsis.

The alterations in circulating IL-6 during sepsis were similar to those seen following a bolus injection of endotoxin. It remains to be determined why the profile of IL-6 differed from that seen for TNF and IL-1. The peak levels of IL-6 at 10 hours correlated with a significant increase of IL-6 release by splenic macrophages. Cyclooxygenase inhibition did not enhance further IL-6 release and, in fact, ibuprofen decreased IL-6 secretion by splenic macrophages from septic animals. This suggests that PGE_2 may be a mediator for the synthesis and release of IL-6.

Although *in vitro* studies do not necessarily reflect the ability and function of cells *in vivo*, there were similarities observed between altered PGE_2 production by splenic macrophages and corresponding PGE_2 plasma levels. The increased production of PGE_2 by splenic macrophages at 5 and 10 hours correlated with significantly elevated levels of PGE_2 in the plasma at the same time points. Because at these time points blockade of cyclooxygenase in splenic macrophage cultures showed an effect on TNF, IL-1, and IL-6 release, our *in vitro* studies may suggest that the immunomodulatory eicosanoid PGE_2 is involved in the regulation of TNF, IL-1, and IL-6 release during sepsis.

The kinetics of the cytokines TNF, IL-1, and IL-6 in plasma, the alterations in circulating PGE_2, and the results obtained in our *in vitro* studies indicate that PGE_2 plays a pivotal role in the cascade of inflammatory responses produced by the interaction of macrophages with bacteria/LPS. The data from our study, together with previous investigations, suggest that LPS may induce the release of cytokines (TNF, IL-1, IL-6) and prostaglandins, which control and regulate the release of each other. The early increase in circulating TNF may result in increased secretion of IL-1, IL-6, and PGE_2, which controls TNF and IL-1 synthesis through a negative feedback mechanism. In the event of an overwhelming release of TNF and IL-1, PGE_2 may downregulate the production of these two cytokines. Although previous studies have demonstrated that PGE_2 has a deleterious effect on cellular immune function after hemorrhage[34] or trauma,[46] one could speculate that PGE_2 also may play a role in dampening those inflammatory responses associated with the excessive secretion of TNF and IL-1 during sepsis.

Acknowledgment

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Lovastatin Inhibits Gallstone Formation in the Cholesterol-fed Prairie Dog

KIMBERLY D. SAUNDERS, M.D., JOE A. CATES, M.D., MOHAMMAD Z. ABEDIN, PH.D., SHEILA REGE, M.D.,
SARKIS F. FESTEKDJIAN, B.A., WARREN HOWARD, B.A., and JOEL J. ROSLYN, M.D.

The efficacy of lovastatin, an inhibitor of hepatic cholesterol synthesis in the prevention of cholesterol gallstone formation, was evaluated in the prairie dog model. Two groups of animals were maintained on either nonlithogenic or 1.2% cholesterol-enriched chow for 21 days. Seven of the animals in each group received lovastatin, and the remaining six received only distilled water. All of the cholesterol-fed/water-treated animals had crystals and 83% had gallstones, but none of the cholesterol-fed/lovastatin-treated animals had gallstones and only three had microscopic crystals. These data indicate that lovastatin inhibits cholesterol gallstone formation in a diet-induced model of gallstone disease.

From the Research and Surgical Services, Sepulveda Veterans Administration Medical Center, Sepulveda, and the Department of Surgery, University of California at Los Angeles School of Medicine, Los Angeles, California

IN 1968, ADMIRAND AND Small[1] demonstrated that the hepatic secretion of cholesterol-saturated bile is a prerequisite to the formation of cholesterol gallstones. The clinical significance of this observation has been expanded by the finding that pharmacologic agents that reduce biliary cholesterol saturation are effective in the dissolution of existing gallstones in select patients.[2–4] Furthermore, with the advent of extracorporeal shock wave lithotripsy treatment for gallstones, these drugs have become increasingly important.[5–7] The efficacy of agents such as ursodeoxycholic acid in dissolving gallstones and fragments ultimately depends on their ability to reduce biliary cholesterol levels, probably *via* multiple mechanisms.[8]

Lovastatin, an inhibitor of hepatic cholesterol synthesis, has been proved to be effective in the treatment of hypercholesterolemia.[9] The drug is orally absorbed, extracted on first pass through the liver, where it exerts its primary effect, and is eliminated almost exclusively *via* biliary excretion. Lovastatin acts by competitively inhibiting HMG-CoA reductase,[8] the rate-limiting enzyme in cholesterol biosynthesis. In clinical trials using both healthy volunteers and patients with hypercholesterolemia, lovastatin administration (80 mg/day, in divided doses) resulted in significant reductions in serum total cholesterol, low-density lipid (LDL) cholesterol, very low-density lipid cholesterol, and triglycerides.[10]

Unlike the previously available cholesterol-lowering agent clofibrate, which increases biliary cholesterol,[11] cholesterol saturation index (CSI), and the incidence of clinical cholelithiasis,[12] HMG-CoA reductase inhibitors have been shown to reduce significantly both biliary cholesterol levels and CSIs in hypercholesterolemic patients[13] and healthy volunteers.[14] Recently Logan and Duane[14,15] demonstrated that combination therapy using lovastatin plus ursodeoxycholic acid resulted in significantly greater reductions in biliary CSI than did ursodeoxycholic acid therapy alone. These preliminary observations suggest that lovastatin may be a useful agent in the treatment of cholesterol gallstones.

The purpose of the present study, therefore, was to evaluate the efficacy of lovastatin in preventing the formation of diet-induced cholesterol gallstones. The prairie dog model was selected for study because its biliary lipid composition has been shown to be similar to that of humans.[16–19] Furthermore cholesterol crystals and gallstones are predictably induced in this model in a time-dependent manner by a diet enriched in cholesterol.[16,17]

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Address reprint requests to Joel J. Roslyn, M.D., Division of General Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

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Materials and Methods

Experimental Design

Adult male prairie dogs (*Cynomys ludovicianus*, trapped in the wild and obtained from Otto Marten Locke of New Braunfels, TX) weighing approximately 1 kg were individually caged in a thermoregulated (23 C) room. Use of the animals followed prior approval by the Veterans Administration Institutional Animal Care and Use Committee. Two groups of 13 animals each were maintained for 21 days on either nonlithogenic control chow (NL) (Purina Laboratory Chow, Ralston-Purina, St. Louis, MO) or 1.2% cholesterol-enriched chow (XOL) (Teklad, Sprague-Dawley, Madison, WI). Both diets contain ample essential fatty acids, minerals, and fiber. Twice a day, following mild sedation (ketamine, 25 mg/kg body weight), seven animals in each group received a 5-mL bolus through an orogastric tube of lovastatin (mevinolin, provided by Merck, Sharp & Dohme, West Point, PA) (1.6 mg/mL dH_2O), while the remaining six received only distilled water.

After a 16-hour fast (water *ad libitum*), each animal was anesthetized with ketamine (100 mg/kg body weight, intramuscularly) and xylazine (1.5 mg/kg body weight, intramuscularly). *Via* a midline laparotomy incision, the cystic duct was exposed, clamped proximally and distally, and divided. A meticulous cholecystectomy then was performed. The distal common bile duct was ligated and a 25-cm silastic catheter (inner diameter, 0.02 inches/outer diameter, 0.037 inches) was placed into the common bile duct. Hepatic bile was collected under mineral oil over ice on an hourly basis for a total of 3 hours. At the termination of the experiment, blood was obtained *via* direct cardiac puncture and serum was prepared. Serum total cholesterol, high-density lipoprotein (HDL), triglycerides, total protein, and albumin were determined using the MONARCH system (I.L. Fisher, Boston, MA).

Analytic Methods

Gallbladder and hepatic bile samples were examined both grossly and microscopically for the presence of gallstones and cholesterol crystals. Fresh bile was centrifuged to remove suspended gallstones and sludge, and the supernatant was aliquoted and subsequently analyzed for levels of cholesterol,[20] phospholipids,[21] total bile acids,[22] and calcium[23,24] using methods that have long been in use in this laboratory. A CSI was calculated using Carey's critical tables.[25]

Statistical Analysis

Statistical comparisons between the dietary groups were made using analysis of variance followed by Tukey's multiple comparison tests. Fisher's exact test was used to compare the incidence of crystals and gallstones among the treatment groups.

Results

Nutritional Parameters

The effects of cholesterol feeding and lovastatin administration on nutritional parameters are shown in Table 1. All animals tolerated the diets and interventions well. Their coats of fur remained healthy and there was no significant diarrhea or other apparent side effects. Serum total protein and albumin were similar among all treatment groups. While body weights decreased slightly ($\leq$ 1%) during the 21 days of the experiment, the trend was uniform throughout all groups. It has been previously noted in this laboratory (unpublished data) that dietary intake of these gregarious animals is slightly higher when they are housed in small groups; hence the necessity of maintaining the prairie dogs in individual cages probably accounts for the minimal decrease in body weight.

Gallstone Formation

Figure 1 summarizes the effects of lovastatin administration on the formation of diet-induced cholesterol gallstones. As expected neither the NL diet group that received water (NL/W) nor the group that received lovastatin developed either gallstones or cholesterol crystals. All of the cholesterol-fed/water animals (XOL/W) had crystals, and 83% (5 of 6 dogs) had gallstones ($p < 0.005$ *versus* NL/W). In contrast none of the cholesterol-fed/lovastatin-treated animals (XOL/LS) had gallstones ($p < 0.005$ *versus* XOL/W), and only three had microscopic crystals ($p < 0.05$ *versus* XOL/W).

Serum Cholesterol and Lipoproteins

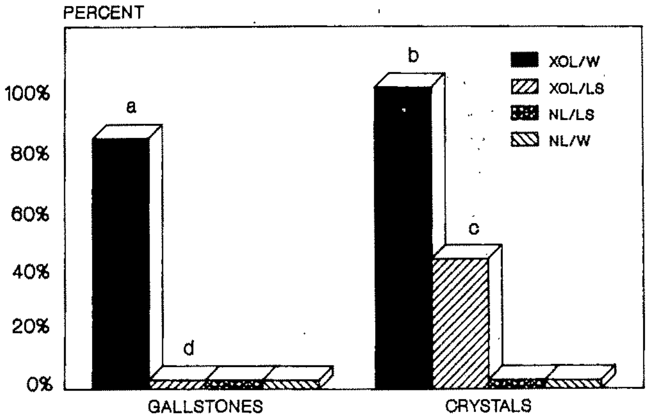
The effects of cholesterol feeding and lovastatin administration on serum cholesterol and lipoprotein levels are shown in Table 2. Cholesterol-fed animals receiving water (XOL/W) had significant elevations in serum total cholesterol ($p < 0.005$), LDL ($p < 0.005$), and HDL ($p < 0.001$) as compared to NL diet-fed animals receiving

TABLE 1. *Nutritional Parameters*

Group	n	% Weight Change	T. Prot* (g/dL)	Albumin* (g/dL)
NL/W	6	1.0 ± 0.2	5.7 ± 0.8	2.4 ± 0.4
NL/LS	7	0.9 ± 0.1	5.7 ± 0.7	2.7 ± 0.4
XOL/W	6	0.9 ± 0.1	6.6 ± 0.7	2.9 ± 0.7
XOL/LS	7	1.0 ± 0.2	6.3 ± 1.0	2.7 ± 0.2

NL, normal diet; W, water-treated (5 mL *via* orogastric tube, twice a day); XOL, 1.2% cholesterol-enriched diet; LS, lovastatin-treated (5 mL of a 1.6 mg/mL solution via orogastric tube, twice a day).

* Serum levels; results are means ± SD.



a p<0.005, b p<0.001 vs NL/W
c p<0.05, d p<0.005 vs XOL/W

FIG. 1. Effect of lovastatin administration on the formation of cholesterol gallstones in prairie dogs. XOL/W = animals fed 1.2% cholesterol-enriched chow and treated with 5 mL water *via* orogastric tube twice a day (positive controls, n = 6); NL/LS = animals fed normal laboratory chow and treated with 8 mg lovastatin in 5 mL water *via* orogastric tube twice a day (negative controls, n = 7); XOL/LS = animals fed 1.2% cholesterol-enriched chow and treated with 8 mg lovastatin in 5 mL water *via* orogastric tube twice a day (experimental group, n = 7). Results represent percent of animals in each group found to have gallstones or cholesterol crystals in their gallbladder bile after 21 days of treatment.

water (NL/W). Cholesterol-fed/lovastatin-treated animals (XOL/LS) demonstrated a 20% to 30% reduction in total cholesterol, LDL, and HDL as compared to cholesterol-fed/water-treated animals (XOL/W). Similarly lovastatin-treated animals on the NL diet (NL/LS) had levels of serum total cholesterol and LDL that were approximately 25% lower than those seen in NL/W animals. While these differences were not significant at the 5% probability level, they were similar in magnitude to reductions in serum lipids seen in human studies with lovastatin.[6]

For further analysis cholesterol-fed/lovastatin-treated animals (XOL/LS) were divided into two groups based on the presence or absence of crystals in their gallbladder bile. When these two groups were compared with each other, significant reductions were seen in total XOL ($p < 0.05$), and LDL ($p < 0.005$) in XOL/LS animals with clear bile, as compared to XOL/LS animals with crystal-containing bile.

Biliary Lipid Composition

Due to the severe extent of gallstone disease in the XOL/W group, available gallbladder bile following centrifugation and gallstone removal was inadequate or unusable for subsequent biochemical analysis. The high prevalence of gallstones and crystals among these animals, however, suggests that gallbladder bile in this group was almost certainly supersaturated with cholesterol. Gallbladder biochemistry for the remaining treatment groups is presented in Table 3. Because the necessary positive control group data were unavailable, no statistical comparisons could be made among treatment groups. Subdivision of the lovastatin-treated animals based on crystal formation, however, revealed that crystal-containing animals had significant elevations in gallbladder bile XOL, phospholipids (PPL), Ca^{2+}, and CSI, as compared to animals with clear bile. Interestingly values for XOL/LS animals with clear bile were remarkably similar to both groups of animals maintained on the NL diets (NL/LS and NL/W).

The effects of lovastatin and cholesterol feeding on hepatic bile composition are shown in Figure 2. Hepatic bile XOL ($p < 0.001$) and PPL ($p < 0.01$) were significantly elevated in XOL/W animals as compared to the NL/W control group. Although hepatic bile CSIs also tended to be higher in the XOL/W animals (1.00 ± 0.34) as compared to the NL/W controls (0.70 ± 0.13), these differences did not achieve statistical significance at the 5% probability level. Lovastatin-treated cholesterol-fed animals (XOL/LS) had significantly reduced hepatic bile XOL levels ($p < 0.05$) than did cholesterol-fed animals that received water (XOL/W). Once again, while hepatic bile CSIs from the XOL/LS group also tended to be lower (0.78 ± 29) than among XOL/W animals (1.00 ± 0.34), these differences were not significant. There were no significant differences between XOL/W and XOL/LS animals with respect to hepatic PPL, total bile acids (TBA), or calcium levels (latter not shown). Administration of lovastatin to animals maintained on control NL chow induced no significant alteration in hepatic bile composition.

Lovastatin-treated cholesterol-fed animals with clear bile had significantly reduced hepatic XOL ($p < 0.001$) and PPL ($p < 0.025$) as compared to those animals that formed crystals (Fig. 3). In fact XOL and PPL levels in the clear bile group closely approximated those found in the NL diet control group. Similarly hepatic bile CSIs from XOL/LS animals with clear bile (0.66 ± 0.14) were very similar to those from the NL control group (0.70

TABLE 2. *Serum Lipid Profile*

Group	XOL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
NL/W	144 ± 57	77 ± 13	36 ± 16
NL/LS	110 ± 30	79 ± 24	27 ± 19
XOL/W	325 ± 71*	139 ± 9†	168 ± 70*
XOL/LS	260 ± 87‡	114 ± 34	130 ± 65‡
Crys	336 ± 11§	128 ± 29	194 ± 30‖
Clear	202 ± 70	103 ± 38	81 ± 23

NL, normal diet; W, water-treated (5 mL *via* orogastric tube, twice a day); XOL, 1.2% cholesterol-enriched diet; LS, lovastatin-treated (5 mL of a 1.6 mg/mL solution *via* orogastric tube, twice a day); crys, subgroup with crystal-containing bile; clear, subgroup with clear bile; results are means ± SD.

* $p < 0.005$. † $p < 0.001$ *vs.* NL/W; ‡ $p < 0.02$ *vs.* NL/LS; § $p < 0.05$; ‖ $p < 0.005$ *vs.* XOL/LS clear.

TABLE 3. *Gallbladder Bile Composition*

Group	XOL (μmol/mL)	PPL (μmol/mL)	TBA (μmol/mL)	Calcium (mg/dL)	CSI
NL/W	6.0 ± 0.9	19.3 ± 5.7	136 ± 15	20.3 ± 4.6	0.72 ± 0.14
NL/LS	5.6 ± 2.2	21.7 ± 5.8	123 ± 34	20.6 ± 5.6	0.69 ± 0.15
XOL/LS	9.8 ± 5.4	32.8 ± 9.9	149 ± 21	27.4 ± 6.6	0.81 ± 0.17
Crys	13.9 ± 6.2*	41.2 ± 8.3*	185 ± 28	33.8 ± 2.0†	0.89 ± 0.23*
Clear	6.6 ± 1.3	26.4 ± 4.9	121 ± 25	22.5 ± 3.5	0.75 ± 0.08

NL, normal diet; W, water-treated (5 mL *via* orogastric tube, twice a day); XOL = 1.2% cholesterol-enriched diet; LS, lovastatin-treated (5 mL of a 1.6 mg/mL solution via orogastric tube, twice a day); crys, subgroup with crystal-containing bile; clear, subgroup with clear bile; results are mean ± standard deviation.

* $p < 0.05$, † $p < 0.01$ *vs.* XOL/LS clear.

± 0.13), whereas CSIs among XOL/LS crystal-containing animals tended to be higher (0.95 ± 0.40) ($p = 0.06$).

Discussion

These data represent the first report of which we are aware documenting the effects of lovastatin on gallstone formation. Using a diet-induced model of cholesterol gallstone disease, we demonstrated that oral administration of lovastatin, an inhibitor of cholesterol biosynthesis, altered biliary lipid composition and inhibited cholesterol gallstone formation. Prairie dogs maintained on a XOL diet for 3 weeks had increased biliary concentrations of cholesterol, phospholipids, and calcium, and furthermore 83% developed cholesterol gallstones. The addition of lovastatin to this potent lithogenic regimen markedly reduced the incidence of cholesterol gallstones in this model. Furthermore no apparent side effects were associated with use of this drug. These findings suggest that lovastatin might also be useful in the dissolution of existing cholesterol gallstones, although this hypothesis remains to be tested.

Many studies have demonstrated that the prairie dog is an animal model uniquely suited to the study of cholesterol gallstone disease.[16-19] Both the biliary lipid composition and the bile acid profile in the prairie dog are remarkably similar to those found in humans. Furthermore, to the extent that the pathogenesis of cholesterol gallstones is understood, the process appears to share similar features in humans and prairie dogs.

In the present study, 21 days of cholesterol feeding resulted in crystal formation in all placebo (XOL/W) animals and gallstones in five of six dogs (83%). In contrast only three of seven (42%) lovastatin-treated, cholesterol-fed animals (XOL/LS) had evidence of crystals, and none had gallstones. Interestingly, among the three XOL/LS animals that did have crystals, serum total cholesterol and

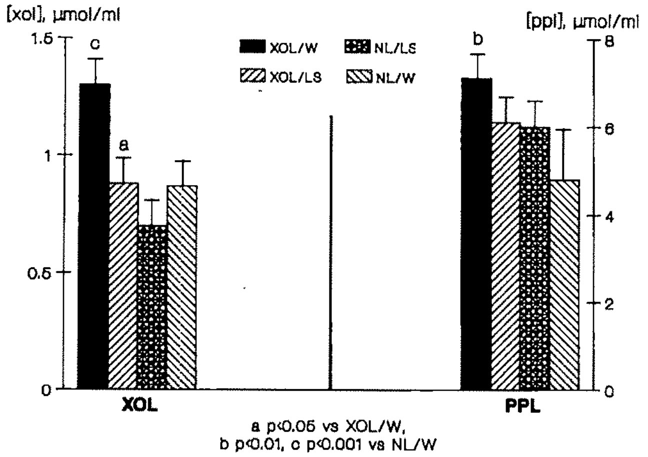


FIG. 2. Effect of lovastatin administration on hepatic biliary lipid composition in cholesterol-fed prairie dogs. XOL = cholesterol; PPL = phospholipids; TBA = total bile acids; XOL/W = animals fed 1.2% cholesterol-enriched chow and treated with 5 mL water *via* orogastric tube twice a day (positive controls, n = 6); NL/W = animals fed normal laboratory chow and treated with 5 mL water *via* orogastric tube twice a day (negative controls, n = 6); XOL/LS = animals fed 1.2% cholesterol-enriched chow and treated with 8 mg lovastatin in 5 mL water *via* orogastric tube twice a day (experimental group, n = 7). Animals were treated for a total of 21 days. Results represent mean ± SEM.

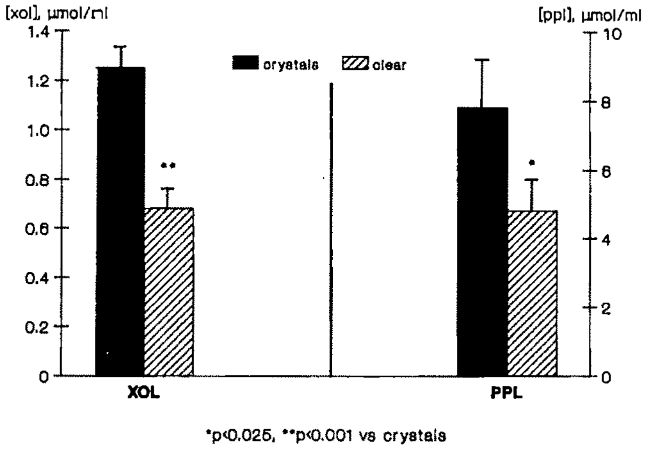


FIG. 3. Comparison of hepatic biliary lipids in cholesterol-fed/lovastatin-treated prairie dogs with clear *versus* crystal-containing gallbladder bile. XOL = cholesterol; PPL = phospholipids; All animals were fed 1.2% cholesterol-enriched chow and treated with 8 mg lovastatin in 5 mL water *via* orogastric tube twice a day for a total of 21 days. Animals were divided into two groups based on the presence (n = 3) or absence (n = 4) of cholesterol crystals in their gallbladder bile. Results represent mean ± SEM.

virtually all gallbladder and hepatic bile parameters measured were typical of untreated cholesterol-fed animals. Furthermore serum and biliary profiles among the four XOL/LS animals with clear bile were similar to those from control animals fed NL chow. These biochemical data, taken together with the gross observations, are consistent with a 'partial response' to lovastatin for the three animals with crystals, and a 'complete response' for the remaining four animals with clear bile. The present study cannot exclude the possibility, however, that lovastatin was effective simply in delaying gallstone formation in this model. Nonetheless this seems improbable given the virtual normalization of biliary biochemistry by lovastatin among animals with clear bile. Rather it seems more likely that the incomplete inhibition of crystal formation seen among lovastatin-treated animals may be due to animal-to-animal variations in gastrointestinal absorption or metabolism of administered drug. Serum lovastatin (mevinolin) levels, which would be most helpful in sorting out these variations, are not available at this time.

The effects of lovastatin administration on serum lipid profile seen in the present study approximate the reductions in serum lipids reported in human studies (using 40 mg; twice a day).[10] Lovastatin administration (8 mg/kg, twice a day) to cholesterol-fed prairie dogs resulted in a 20% to 30% reduction in serum total cholesterol, LDL, HDL, and triglycerides as compared to animals that received no drug. Similarly lovastatin-treated animals fed the control NL diet also showed a 25% decrease in serum total cholesterol and LDL compared to nonlovastatin-treated controls. Because fur coat appearance, body weight, and serum total protein and albumin were all similar among the lovastatin- and nonlovastatin-treated groups, observed differences in serum lipid profile presumably reflect a specific effect of the drug rather than a nonspecific perturbation in dietary intake.

It is impossible to define precisely the mechanism by which lovastatin exerts its inhibitory effect on gallstone formation based on the findings in the present experiment. We would speculate, however, that the observed inhibition of cholesterol crystal and gallstone formation resulted from a reduction in hepatic synthesis of cholesterol. Lovastatin directly inhibits the activity of HMG-CoA reductase,[8] the rate-limiting enzyme in cholesterol biosynthesis (Fig. 4). Furthermore studies by Stone and colleagues[26] using an *in vivo* rat model demonstrated inhibition of both hepatic cholesterol synthesis and secretion after lovastatin infusion, with no effect on bile flow, bile salt output, or phospholipid output. In the present study, lovastatin significantly reduced hepatic biliary cholesterol concentration without altering phospholipid, total bile acid, or calcium levels. These findings are consistent with, but do not prove, the hypothesis that inhibition of gallstone formation by lovastatin may be due to reduction in cholesterol secretion by the liver. Additional studies, specifically designed to examine the effects of lovastatin on cholesterol synthesis and secretion, are necessary to clarify this mechanism further.

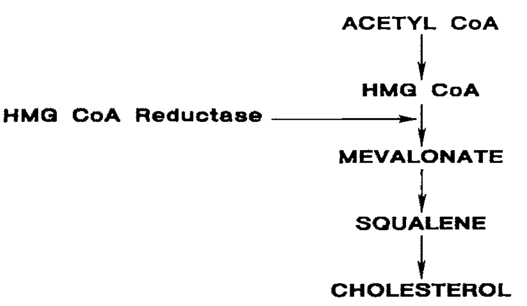


FIG. 4. Simplified version of the cholesterol biosynthetic pathway. HMG-CoA reductase, which converts HMG-CoA to mevalonate, is the rate-limiting enzyme in this sequence.

Data from our animal studies indicate that lovastatin, which suppresses cholesterol biosynthesis, inhibits cholesterol gallstone formation in a diet-induced model of gallstone disease. Studies are currently underway to define further the mechanism by which gallstone inhibition is achieved and to explore the possible role of lovastatin in gallstone dissolution.

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Dose Response of Fish Oil *Versus* Safflower Oil on Graft Arteriosclerosis in Rabbit Heterotopic Cardiac Allografts

KWOK L. YUN, M.D.,* JAMES I. FANN, M.D.,* MITCHELL H. SOKOLOFF, B.A., LOREN G. FONG, PH.D.,‡
GEORGE E. SARRIS, M.D.,* MARGARET E. BILLINGHAM, M.B., M.D.,† and D. CRAIG MILLER, M.D.*

From the Department of Cardiovascular Surgery and Pathology,† Stanford University School of Medicine, Stanford, and the Research Institute of the Palo Alto Medical Foundation,‡ Palo Alto, California*

With the advent of cyclosporin A, accelerated coronary arteriosclerosis has become the major impediment to the long-term survival of heart transplant recipients. Due to epidemiologic reports suggesting a salutary effect of fish oil, the dose response of fish oil on graft coronary arteriosclerosis in a rabbit heterotopic cardiac allograft model was assessed using safflower oil as a caloric control. Seven groups of New Zealand White rabbits (n = 10/group) received heterotopic heart transplants from Dutch-Belted donors and were immunosuppressed with low-dose cyclosporin A (7.5 mg/kg/day). Group 1 animals were fed a normal diet and served as control. Group 2, 3, and 4 animals received a daily supplement of low- (0.25 mL/kg/day), medium- (0.75 mL/kg/day), and high- (1.5 mL/kg/day) dose fish oil (116 mg n-3 polyunsaturated fatty acid/mL), respectively. Group 5, 6, and 7 animals were supplemented with equivalent doses of safflower oil (*i.e.*, 0.25, 0.75, and 1.5 mL/kg/day). Oil-supplemented rabbits were pretreated for 3 weeks before transplantation and maintained on the same diet for 6 weeks after operation. The extent of graft coronary arteriosclerosis was quantified using computer-assisted, morphometric planimetry. When the animals were killed, cyclosporin A was associated with elevated plasma total cholesterol and triglyceride levels in the control group. While safflower oil prevented the increase in plasma lipids at all dosages, fish oil ameliorated the cyclosporin-induced increase in total cholesterol only with high doses. Compared to control animals, there was a trend for more graft vessel disease with increasing fish oil dose, as assessed by mean luminal occlusion and intimal thickness. A steeper trend was observed for increasing doses of safflower oil; compared to the high-dose safflower oil group, animals supplemented with low-dose safflower oil had less mean luminal occlusion (16.3% ± 5.9% *versus* 41.4% ± 7.6%, $p < 0.017$) and intimal thickness (7.9 ± 1.9 μm *versus* 34.0 ± 13.0 μm, analysis of variance: $p = 0.054$). Low-dose safflower oil also had a slight, but nonsignificant, beneficial effect on graft vessel disease when compared to control rabbits. The same trends were observed in the degree of histologic rejection (0 = none to 3 = severe) in fish oil- and safflower oil-treated animals. Rejection score correlated weakly but significantly ($p = 0.0001$) with mean luminal occlusion ($r = 0.52$) and intimal thickness ($r = 0.46$). Therefore allograft coronary disease in this model appeared to exhibit an unfavorable, direct-dose response to fish oil and safflower oil, independent of effects on plasma lipids. Further controlled experiments in animals and humans are needed to determine the utility and safety of polyunsaturated fatty acid supplementation in the prevention of accelerated human cardiac allograft vessel disease.

SHORT-TERM (1 year) survival after heart transplantation has increased from 22% in 1969 to 84% in 1985.[1] While many factors have contributed (such as improved immunosuppressive protocols and early rejection management), the introduction of cyclosporin A (CyA) was accompanied by significant improvements in cell-mediated rejection rates and early survival. With increased late survival, accelerated graft coronary arteriosclerosis (GCA) has become the major impediment to long-term survival.[2,3] Findings of GCA were noted in the early days of transplantation in canine cardiac allografts[4] and also clinically.[2,5] Graft coronary arteriosclerosis[3] is characterized by diffuse concentric myointimal proliferation without significant damage to the internal elastic lamina, affecting both large epicardial and smaller intramyocardial coronary arteries. It was postulated that chronic rejection results in immune-mediated endothelial injury followed by myointimal proliferation and lipid accumulation from influx of low-density lipoprotein.[5] The precise mechanisms of GCA, however, remain unclear.

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Address reprint requests to D. Craig Miller, M.D., Department of Cardiovascular Surgery, Cardiovascular Research Center, Stanford University Medical Center, Stanford, California 94305.

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Because of the possible role of platelets in this process, aspirin and dipyridamole were administered prophylactically[3,6] in an attempt to prevent GCA. However this regimen proved to be insufficient because the incidence of GCA, despite improved immunosuppression with the advent of CyA, remains 40% to 50% after 3 to 5 years.[2,3,6–8] Clinically this disease is silent (no angina pectoris) and can lead to myocardial infarction, congestive heart failure, and sudden death. Because of the diffuse nature of GCA, the only current successful therapy is retransplantation (58.8% of retransplant procedures in one report). Clearly, with the paucity of heart donors, better approaches to understanding and preventing GCA are needed.

A protective effect of high-dose dietary fish oil on coronary artery disease has been suggested in epidemiologic studies.[9,10] Fish oil also has been shown to inhibit arteriosclerosis in atherosclerosis-prone Japanese quail,[11] canine venoarterial autografts,[12,13] small-diameter prosthetic arterial grafts,[14] abrasion-induced coronary artery disease in hypercholesterolemic swine,[15] and aortic disease in cholesterol-fed rabbits[16,17] and monkeys.[18] Positive results, however, were not demonstrated in all experiments. Fish oil did not have a beneficial effect on myointimal proliferation in canine venoarterial allografts[19] or aortic arteriosclerosis in Watanabe heritable hyperlipidemic rabbits[20,21]; in other studies, fish oil may actually have exerted a deleterious effect.[22,23] Despite these many investigations, the mechanism(s) of effects of omega-3 fatty acids, the putative active ingredient in fish oil, on the pathogenesis of arteriosclerosis have not been elucidated.[24,25] Other polyunsaturated fatty acids, such as linoleic acid, also have been suggested to have an immunosuppressive[26,27] and possibly an anti-atherogenic effect.[17,28]

Recently fish oil was shown to inhibit accelerated GCA in a rat heterotopic cardiac allograft model.[29] Important differences in the histopathology of the rat coronary lesions compared to humans were noted, specifically, occasional extensive injury involving the elastic tissue of the media. Furthermore marked differences in lipid and prostanoid metabolism exist between rat and humans (resistance to hypercholesterolemia and no synthesis of PGI_3 in rats), and lipid and prostaglandin levels were not measured. In addition large doses of fish oil were used and no dose-response information was obtained. Because of the comparatively rapid development of GCA in the rabbit, the remarkable similarities in rabbit GCA histopathology compared to humans,[30] and the relatively greater similarities in lipid metabolism between rabbit and humans, we assessed the dose response of fish oil *versus* safflower oil (predominantly polyunsaturated linoleic acid [C18:2n-6]) on GCA in rabbit heterotopic cardiac allografts immunosuppressed with low-dose CyA.

Materials and Methods

Experimental Protocol

Major histocompatibility complex (MHC) mismatched Dutch-belted and New Zealand White (NZW) rabbits weighing 1.6 to 4.2 kg (Rabbit Barn, Turlock, CA) were used as donors and recipients, respectively. All animals were maintained on standard Purina rabbit chow (Mannapro, Stockton, CA). Seventy NZW rabbits were divided equally into seven groups. Group 1 served as control. Animals in groups 2, 3, and 4 received a daily supplement of low- (FO[1], 0.25 mL/kg/day), medium- (FO[m], 0.75 mL/kg/day), and high- (FO[h], 1.5 mL/kg/day) dose cholesterol-free fish oil (emulsified fish oil concentrate [TwinLabs, Ronkonkona, NY]; each 15-mL dose contains 1024 mg eicosapentaenoic acid [EPA] and 712 mg docosahexaenoic acid [DHA]), respectively. These dose levels correspond roughly to human consumption of 2, 6, and 12 g, respectively, of omega-3 fatty acids daily. To control for the caloric content of the fish oil, groups 5, 6, and 7 were supplemented with equivalent doses of safflower oil (rich in linoleic acid; Saffola Quality Foods, Los Angeles, CA); *i.e.*, 0.25 (SO[1]), 0.75 (SO[m]), and 1.5 mL/kg/day (SO[h]).

Before dietary manipulation appropriate baseline blood samples were drawn for measurement of plasma cholesterol, triglyceride, and lipoprotein profile, low-density lipoprotein (LDL) isolation, copper oxidation potential of LDL, and serum thromboxane B_2 (TxB_2) levels. Fish oil and safflower oil dietary supplementation (except for the control group) were started 3 weeks before transplantation. Six weeks after operation, blood was drawn for repeat measurement of all biochemical parameters and the allografts were harvested for quantitative morphometric analysis of GCA and assessment of rejection.

Operation

Briefly, the animals were anesthetized with 0.75 to 1.0 mL/kg of 'rabbit cocktail' (ketamine chloride, 50 mg/mL; xylazine [Rompun], 4 mg/mL; acepromazine maleate, 1 mg/mL; 15% v/v 0.9% NaCl) supplemented with 0.5% to 1.0% halothane through a nose cone. All recipients received prophylactic intravenous cefamandole (200 mg) before abdominal incision. The abdominal aorta and vena cava were dissected free, taking special caution to preserve lumbar branches. The donor was then given 1000 units of heparin intravenously and *via* a midline laparatomy, the inferior vena cava was transected to empty the heart. After performing a sternotomy, the ascending aorta and pulmonary artery were divided, the pulmonary veins and venae cavae were ligated, and the beating heart was harvested and quickly arrested and cooled by immersion in iced saline. The pulmonary artery of the donor was anastomosed end-to-side to the recipient's infrarenal vena cava using continuous polypropylene (7-0) suture followed by a similar end-to-side anastomosis between the donor's aorta and the recipient's infrarenal aorta. Allografts were cooled continuously with iced saline during the procedure. Only hearts recovering fully with vigorous contraction within 10 minutes of reperfusion were included in the study (3% were excluded on this basis).

All rabbits were treated with 7.5 mg/kg/day intramuscular cyclosporin A (CyA), beginning immediately after operation. After 6 weeks, under deep halothane anesthesia, both donor and recipient hearts were harvested. All allografts had visible mechanical activity when the animals were killed.

All animals received humane care in compliance with *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (DHEW [NIH] Publication 85-23, revised 1985). The study was approved by the Stanford Laboratory Animal Review Committee and conducted according to university policy.

Quantitative Morphometric Analysis

The donor and recipient hearts were fixed in 10% formalin and both ventricles divided into basal, middle, and apical slices. The middle slice was embedded in paraffin for sections with hematoxylin and eosin and elastin Van Gieson staining to assess the degree of rejection and to identify the internal (IEL), respectively. Microscopic sections stained with elastin Van Gieson stain were individually magnified and projected, and the perimeters of the lumen and IEL for all coronary arteries visualized were traced. Tangentially cut vessels were not included to avoid errors in tracing the various perimeters. Each coronary artery tracing was digitized with a Hewlett-Packard (H-P) 9111A digitizer tablet and analyzed using the appropriate magnification factor on a H-P 9310 computer workstation (Hewlett-Packard, Downers Grove, IL) with software developed in our laboratory. The perimeters and areas of the lumen (P_L, A_L) and the IEL (P_{IEL}, A_{IEL}) were computed by direct integration. Luminal area corrected to a perfect circle (LA_c), intimal area (IA), and percentage luminal occlusion (%LO) were calculated by the following formula:

$$LA_c = P_L^2/4 \times \pi$$

$$IA = A_{IEL} - A_L$$

$$\%LO = (IA/[IA + LA_c])$$

The average intimal thickness (IT) for each coronary artery was computed directly using an algorithm that determined the distance between the IEL and lumen around the entire coronary perimeter. Intimal thickness was measured in μm and LA_c and IA were measured in μm^2. For each heart the following additional morphometric variables were calculated:

Number of vessels digitized (N)

Mean percent luminal occlusion ($\%LO_m$; [sum of all %LO]/N)

Corrected mean percent luminal occlusion weighed by vessel size

($\%LO_c$; sum of all IA/[sum of all IA + sum of all LA_c])

Rejection Score

Rejection was graded blindly by one experienced cardiac pathologist (ME Billingham) on a scale of 0 to 3 according to the Billingham rejection scale[31] (Table 1).

Blood Sample Preparation

Blood was drawn from the central ear artery *via* a 19-gauge needle while the animal was tranquilized with rabbit cocktail (0.5 mL/kg subcutaneously). Samples were processed as follows: 15 mL of blood was drawn into ethylene diamine tetraacetic acid (EDTA) tubes and centrifuged at 1500*g* for 30 minutes at 4 C. The supernatant plasma was collected into fresh glass tubes for lipid determination. Serum samples were prepared from 15 mL of blood drawn into unsiliconized clotting tubes with serum separator (SST tubes, Becton-Dickinson, Rutherford, NJ). The tubes were incubated for 1 hour in a 37 C water bath and then centrifuged at 2000*g* for 30 minutes at 4 C. The supernatant serum was transferred into Eppendorf tubes, snap frozen, and stored at −70 C until assayed for TxB_2 levels.

Plasma Lipids

The following plasma lipids and lipoprotein subclasses were measured: Total cholesterol (TC), triglycerides (TG), very low-density lipoprotein (VLDL), low-density lipo-

TABLE 1. *Billingham Grading Scale for Assessment of Degree of Rejection*

Grade	Histologic Appearance
0	No rejection
1	Mild rejection: early perivascular and endocardial lymphocytic infiltrate with endocardial and interstitial edema
2	Moderate rejection: increased interstitial, perivascular, and endocardial lymphocytic infiltrate with focal myocyte damage
3	Severe rejection: diffuse and massive lymphocytic infiltrate with myocyte necrosis and interstitial hemorrhage

protein (LDL), total high-density lipoprotein (HDL), and HDL_2 and HDL_3 subfractions. Total cholesterol was determined using a standard enzymatic package (Boehringer-Mannheim Diagnostics, Houston, TX) with techniques developed by Allain,[32] Roexchlau,[33] and Trinder[34]; TG was determined using the A-Gent Triglyceride Test with a single-step enzymatic technique (microbial lipase, Abbott, Santa Fe Springs, CA)[35]; HDL by a dextran sulfate precipitation procedure[36]; and HDL_2 and HDL_3 subclasses with a modification of the Gidez technique.[37] Very low-density lipoprotein and LDL were calculated as follows: VLDL = TG/5 and LDL = TC − VLDL − HDL. All plasma lipid and lipoprotein levels are expressed as mg/dL.

Serum Thromboxane Assay

Thromboxane A_2 level was measured by radioimmunoassay for its stable metabolite, TxB_2, in duplicate using tritiated TxB_2 tracer and rabbit anti-TxB_2 antiserum (Dupont Products, North Billerica, MA). Standard curves were constructed for each assay with known amounts of TxB_2 (10, 25, 50, 100, 250, 500, and 1000 pg/0.1 mL). Values were divided by 100 and expressed as nanograms per milliliter of serum.

LDL Preparation

Low-density lipoprotein was prepared from citrated plasma and isolated by density ultracentrifugation (d = 1.019 to 1.063 g/mL) with EDTA added. Isolated LDL preparations were dialyzed against phosphate-buffered saline for 24 hours at 4 C with three buffer changes. Protein was determined by a modification the method of Lowry[38] using bovine serum albumin as a standard.

LDL Copper Oxidation (Cu^{++} Ox) Potential

Low-density lipoprotein oxidation potential was determined by incubating isolated LDL (100 μg/mL) supplemented with 10 μmol/L $CuSO_4$ for 24 hours at 37 C in a CO_2 incubator. Parallel incubation in the absence of Cu^{++} was performed for basal oxidation level. Extent of lipid peroxidation was measured as malondialdehye (MDA) equivalents by thiobarbituric acid reactive substance (TBARS),[39] which is expressed as nanomoles MDA equivalent/mg protein.

Fatty Acid Analysis

Fatty acid contents of fish oil and safflower oil were determined on a Varian 3700 gas chromatograph using a 6-foot glass column (SP 2330 packing, Supelco Inc., Bellafonte, PA) with temperature programming from 170 to 230 C at 2 C/minute. Reference standards for fatty acid analysis were products of Sigma Chemical Company (St. Louis, MO).

Statistical Analysis

Data are expressed as mean ± 1 standard error of the mean. For rejection score (RS) and each morphometric variable, the dose response to fish oil or safflower oil compared to control group was assessed separately using one way analysis of variance (ANOVA); a F statistic corresponding to $p < 0.05$ was considered statistically significant. If a significant difference was detected by ANOVA, a multiple comparison procedure (Fisher protected least significant difference [PLSD] test) was used to determine which individual comparisons were significant. A p value less than 0.017 (Bonferonni correction for using the same control group in evaluating the dose response to both fish oil and safflower oil, and for comparison to other concurrent drug treatment groups) was considered to be significant. Similarly one-factor ANOVA was performed to assess the differences among all seven groups in terms of ischemic time, number of coronary vessels digitized, percentage weight gain, and LDL oxidation level (with and without copper) followed by Fisher PLSD multiple comparison test if a significant difference was detected ($p < 0.025$ considered to be significant [*vide supra*], except for LDL oxidation level, where $p < 0.05$ was considered significant). Differences between isocaloric fish oil and safflower oil groups, and changes between baseline and sacrifice values were assessed with unpaired and paired t tests, respectively ($p < 0.05$ considered significant). Probability values ≤ 0.1 but ≥ 0.05 were considered to be possibly significant and are reported as such.

Results

The measured fatty acid contents of fish oil and safflower oil are shown in Table 2. While omega-3 fatty acids (EPA + DHA) account for 34% of the fatty acids in fish oil, linoleic acid accounts for 80% of safflower oil. Although fish oil has a higher double-bond index (DBI = sum of percentage of each unsaturated fatty acid multiplied by its number of double bonds) than safflower oil (243.3 *versus* 171.3), the polyunsaturated/saturated fat (P/S) ratio is more than five times higher in safflower oil (9.75 *versus* 1.94).

There were no significant differences between the groups in terms of percentage weight gain (ANOVA: F = 1.236, p = 0.30; Fig. 1) or number of coronary vessels per graft analyzed (ANOVA: F = 0.895, p = 0.50; Table 3). The differences in ischemic time were marginally significant (ANOVA: F = 2.199, p = 0.053; Table 3). Based on multiple comparison analysis, ischemic time was significantly longer in FO(m) than FO(h), FO(l), SO(l), and

TABLE 2. *Measured Composition of the Fish Oil and Safflower Oil Dietary Supplements*

Fatty Acids	Fish Oil	Safflower Oil
C14:0	6.15	—
C16:0	16.01	6.32
C16:1n-7	10.12	—
C18:0	3.18	1.84
C18:1n-9	15.41	12.09
C18:2n-6 (linoleic acid)	4.67	79.60
C18:3n-3	4.52	—
C18:4n-3	3.25	—
C20:4n-6	3.39	—
C20:5n-3 (EPA)	21.29	—
C22:6n-3 (DHA)	11.99	—
P/S ratio	1.94	9.75
DBI	243.3	171.3

Values are given in percentage of total fatty acids.
P/S, polyunsaturated/saturated fat ratio; DBI, double bond index.
Each 15 mL of fish oil and safflower oil contains 5.4 IU and 15 IU of vitamin E, respectively.

control groups. It should be noted, however, that most of the animals in this group by chance underwent transplantation at the beginning of the study.

Histopathology

The histopathologic appearance was one of diffuse GCA with lesions in vessels of all sizes; *i.e.*, medium and small intramyocardial vessels as well as large epicardial vessels. Furthermore %LO was, for most grafts, higher for smaller vessels. The lesions were characteristically concentric with subintimal proliferation consisting largely of transformed smooth muscle cells and macrophages containing a small lipid component (foam cells). The media was not usually involved in the process, with an essentially intact overlying internal elastic lamina. A representative lesion is illustrated in Figure 2.

Morphometric Analysis

There was no evidence of coronary artery disease in all recipient hearts. The results of the quantitative morphometric assessment of the extent of accelerated GCA in the donor allograft hearts are summarized in Table 3. The dose responses to fish oil and safflower oil compared to control rabbits were evaluated separately. Although statistically insignificant by ANOVA, there was a trend for more GCA with increasing fish oil supplementation, as assessed by all three parameters of graft vessel disease. A similar, but steeper, trend was observed for increasing doses of safflower oil. The animals receiving low-dose safflower oil tended to have less GCA compared to the control group (although the difference was not statistically significant). As indexed by %LO_m, SO(h) developed more disease relative to SO(l). The differences in %LO_c and IT were marginally significant (ANOVA: F = 2.814, p = 0.054 and F = 2.736, p = 0.059, respectively). Using a multiple comparison test, SO(l) again had significantly less GCA than SO(h). When comparing paired isocaloric groups of fish oil and safflower oil, there was a tendency for less GCA in rabbits receiving lower doses of safflower oil (Fig. 3). In the low-dose range, safflower oil may be more beneficial than fish oil in terms of %LO_c (8.5% ± 2.8% *versus* 20.8% ± 5.8%, p = 0.07) and IT (7.9 ± 1.9 μm *versus* 18.0 ± 5.1 μm, p = 0.086).

Rejection

Dose response to fish oil and safflower oil in terms of rejection is depicted graphically in Figure 4. Although insignificant, the same trends were observed in the degree of rejection as for GCA; *i.e.*, more rejection with higher doses of fish or safflower oil. When all grafts were combined, GCA correlated directly and significantly with rejection score (%LO_m *versus* RS, R = 0.52, p = 0.0001; %LO_c *versus* RS, R = 0.52, p = 0.0001; IT *versus* RS, R = 0.46, p = 0.0001), but the correlations were weak. Safflower oil groups had lower RS (but not significantly so) than did the fish oil groups at all dosage levels.

Plasma Lipids and Lipoprotein Profile

Lipid data are summarized in Table 4. The pretreatment baseline cholesterol level tended to be lower in the control group (ANOVA; p = 0.11), primarily due to a significantly lower level of HDL_3 ($p < 0.05$). Other lipid parameters were comparable. CyA significantly increased both TC (p = 0.0001) and TG (p = 0.007) in the control

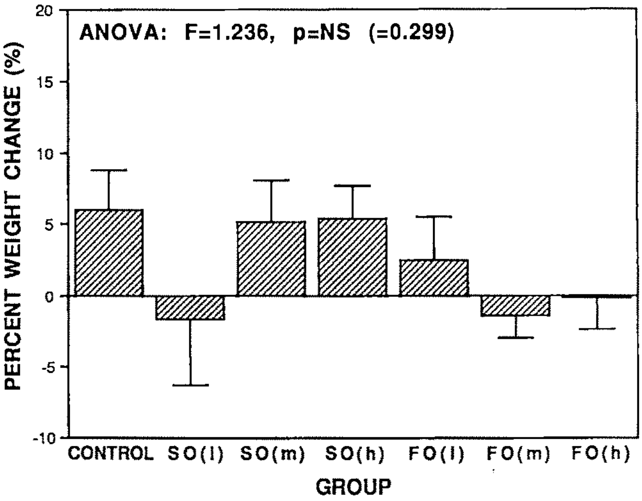


FIG. 1. Graphic summary of percentage weight change. See text for definitions of group abbreviations. ANOVA = analysis of variance; multiple comparisons were performed using the Fisher PLSD test. Values are expressed as mean ± 1 SEM.

TABLE 3. *Summary of Graft Ischemic Time and Quantitative Morphometric Analysis of Accelerated Graft Coronary Arteriosclerosis in the Control Group and the Six Dietary Fat Treatment Groups*

Group	Ischemic Time (min)	Number of Vessels per Graft (n)	Mean Luminal Occlusion (%)	Corrected Mean Luminal Occlusion (%)	Intimal Thickness (μm)
Control	21.2 ± 1.1	21.9 ± 2.5	22.9 ± 5.9	12.3 ± 4.5	9.3 ± 3.6
SO(l)	20.2 ± 0.8	19.3 ± 2.5	16.3 ± 3.7	8.5 ± 2.8	7.9 ± 1.9
SO(m)	22.1 ± 1.2	20.0 ± 2.7	24.7 ± 5.6	15.1 ± 4.0	13.0 ± 3.5
SO(h)	22.6 ± 1.0	22.2 ± 2.4	41.4 ± 7.6*	29.1 ± 8.4†	34.0 ± 13.0‡
FO(l)	20.3 ± 0.4	20.0 ± 12.1	27.7 ± 5.8	20.8 ± 5.8‖	18.0 ± 5.1¶
FO(m)	24.9 ± 1.6§	14.8 ± 5.2	31.4 ± 5.7	22.6 ± 5.8	28.0 ± 8.1
FO(h)	21.3 ± 1.0	20.2 ± 6.3	36.1 ± 5.2	31.5 ± 7.1	26.0 ± 6.5

* ANOVA: F = 3.492, p = 0.026; p < 0.017 *vs.* SO(l).

† ANOVA: F = 2.814, p = 0.054; p < 0.017 *vs.* SO(l).

‡ ANOVA: F = 2.736, p = 0.059; p < 0.017 *vs.* SO(l).

§ ANOVA: F = 2.199, p = 0.053; p < 0.025 *vs.* Control, SO(l), FO(l), FO(m).

Student's paired t test: ‖ p = 0.07 *vs.* SO(l); ¶ p = 0.086 *vs.* SO(l).

Values are expressed as mean ± 1 SEM.

group. This was reflected by elevations of LDL (p = 0.005) and VLDL (p = 0.006), respectively, with no change in HDL (nor HDL subclasses). Consequently the TC/HDL ratio was higher at sacrifice (6.4 ± 1.0 *versus* 2.1 ± 0.1, p = 0.002; Table 4). Conversely safflower oil prevented this CyA induced increase in lipids at all dosages. High-density lipids decreased in SO(m) over time, primarily due to lower HDL_2 levels. In contrast, while effective in preventing increases in TG and VLDL at all dosages, fish oil reduced TC and LDL only at high dose, thereby pre-

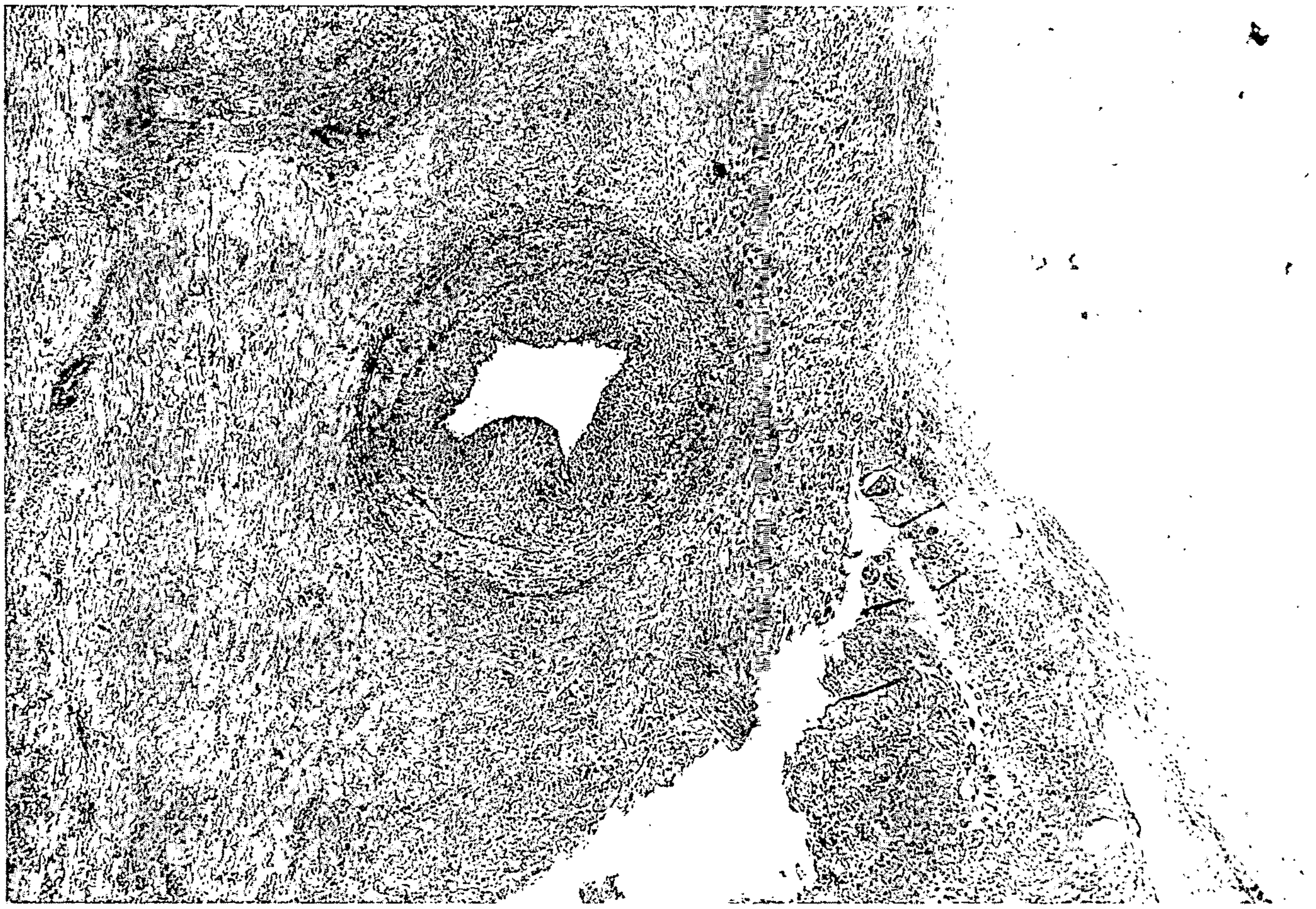


FIG. 2. Representative graft coronary arteriosclerotic lesions characterized by concentric subintimal proliferation with the internal elastica mostly intact (Elastic von Gieson, original magnification ×100).

venting an increase in the TC/HDL ratio. At this fish oil dose, however, there was a concomitant decrease in HDL (HDL_3), and a marginal increase in TG and VLDL.

Serum Thromboxane

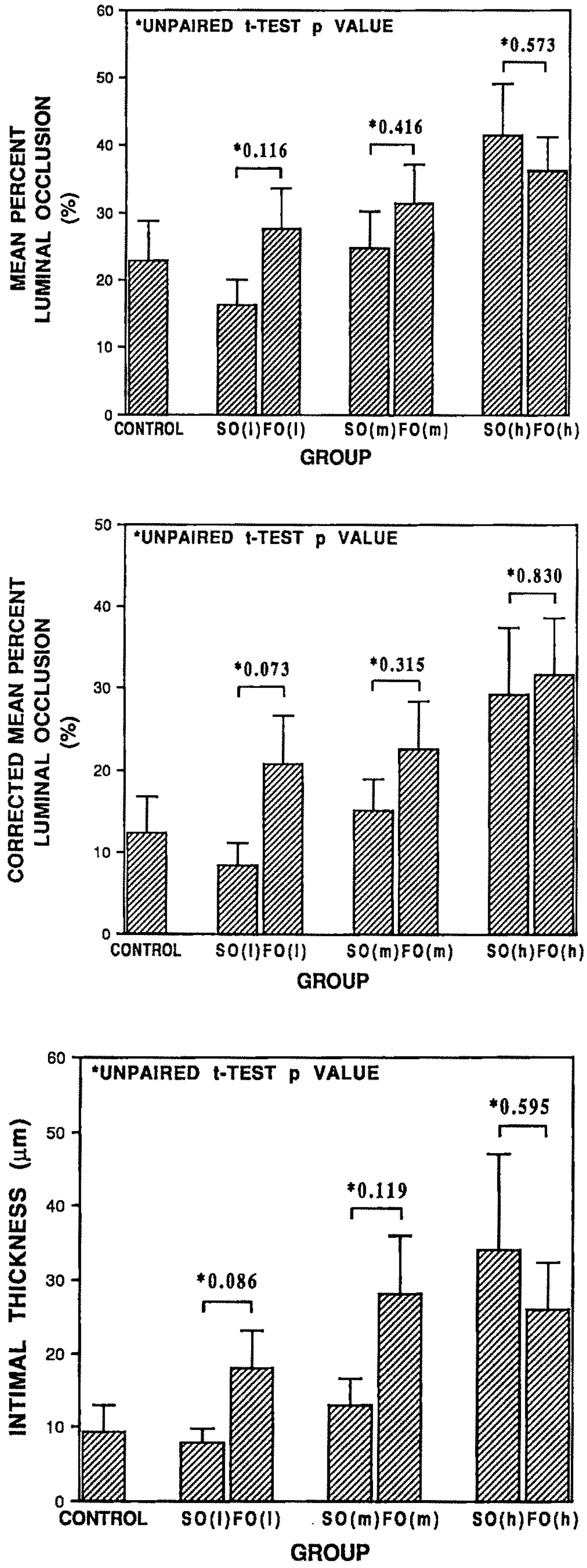
Serum TxB_2 response in all groups was highly variable, and no significant changes from baseline values were detected in any of the groups (Fig. 5). Furthermore there was no correlation between all three GCA parameters and TxB_2 level when the animals were killed ($\%LO_m$ *versus* TxB_2, R = 0.114, p = 0.37; $\%LO_c$ *versus* TxB_2, R = 0.026, p = 0.84; IT *versus* TxB_2, R = 0.158, p = 0.20) or percentage changes in TxB_2 ($\%LO_m$ *versus* TxB_2 % change, R = 0.037, p = 0.76; $\%LO_c$ *versus* TxB_2 % change, R = 0.050, p = 0.69; IT *versus* TxB_2 % change, R = 0.013, p = 0.92).

LDL Oxidation Potential

Low-density lipoprotein peroxidation levels, as measured by TBARS, with and without the addition of copper are summarized in Table 5. Baseline LDL oxidation levels were significantly higher in FO(h) and FO(l). With Cu^{++} oxidation potentials were higher, but remained relatively constant in all safflower oil-supplemented groups and the control group. In contrast LDL peroxidation was markedly increased in all fish oil groups. A direct dose response to fish oil was seen, with FO(h) oxidation potential being significantly higher than that for FO(m) and FO(l).

Discussion

In this study we chose 29, 87, and 174 mg/kg/day of omega-3 fatty acids to be low-, medium-, and high-dose fish oil, respectively, to evaluate the dose response of fish oil on GCA. The low dose is equivalent to human consumption of approximately 2 g per day (70 kg for humans), which is the recommended manufacturer's dose. Eskimos consume approximately 5 to 10 g of EPA and DHA per day[25]; our medium and high fish oil doses correspond to the lower and upper ends of this range, respectively. The amounts of fish oil selected, therefore, represent clinically as well as historically relevant dosages. Although not statistically different among the groups, it should be noted



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FIGS. 3A–C. Comparison of quantitative morphologic extent of accelerated graft coronary arteriosclerosis between isocaloric fish oil and safflower oil treatment groups in terms of mean percent luminal occlusion ($\%LO_m$, A), corrected mean percent luminal occlusion ($\%LO_c$, B), and intimal thickness (IT, C). Compared to fish oil at low dose, safflower oil may possible reduce graft vessel disease in terms of $\%LO_c$ and IT. Control group values are also shown as standards. See Table 3 for details of the statistical analysis and text for definitions of group abbreviations. Values are expressed as mean ± 1 SEM.

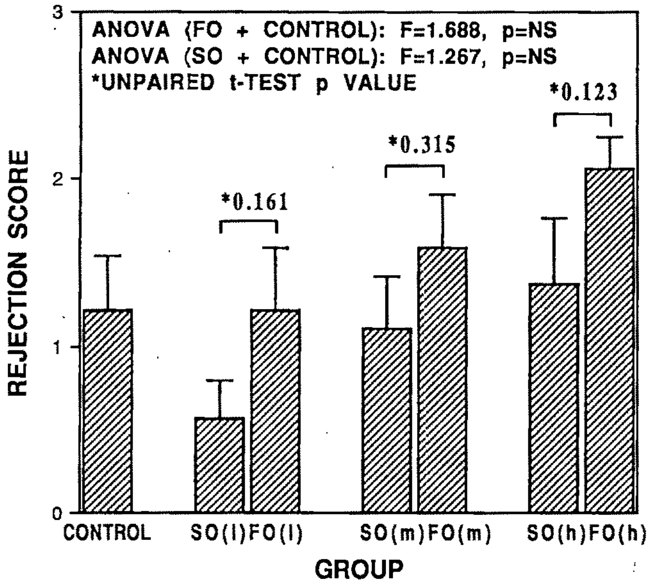

FIG. 4. Comparison of rejection scores between isocaloric fish oil and safflower oil treatment groups. Although statistically insignificant, a trend exists toward greater rejection with higher dietary fat supplement dose. At isocalaric doses, safflower oil appears to reduce rejection although not significantly. See text for detailed discussion and definitions of group abbreviations. ANOVA = analysis of variances. Values are expressed as mean ± 1 SEM.

that most animals gained minimal (less than 5%) or no weight during the course of the study (Fig. 1). This was possibly due to weight loss in the catabolic early postoperative period.

Morphometric Analysis

In this study, because the corrected mean percentage of luminal occlusion was calculated by dividing the sum of all IA by the sum of all IA plus sum of all LA_c for each heart, it follows that larger vessels with greater luminal area would contribute more to $\%LO_c$ than $\%LO_m$. In contrast $\%LO_m$ had equal contribution from vessels of all sizes. Hence $\%LO_m$ and $\%LO_c$ represented indicators of small- and large-vessel disease, respectively. The data, therefore, implied that GCA predominated in small- and medium-sized vessels ($\%LO_m$ more than $\%LO_c$ in the control group). Neither fish oil nor safflower oil affected the relative distribution of lesions. The results also indicated a possible direct dose response of dietary fish oil and safflower oil on GCA as reflected by $\%LO_m$, $\%LO_c$, and IT; *i.e.*, higher oil dosages were associated with more graft vessel disease (this trend with fish oil did not reach statistical significance due to high variability). The dose response to safflower oil, in contrast, demonstrated a steeper trend with significantly more GCA in the SO(h) than SO(l) group. The data also suggested that low-dose safflower oil may inhibit the development of GCA in this CyA-treated rabbit model.

Sarris et al.[29] recently demonstrated a beneficial effect of fish oil (compared to safflower oil) on GCA in a rat heterotopic cardiac transplant model, using a dose containing 21% more omega-3 fatty acids than the highest fish oil dose in this study. These seemingly contradictory results may be explained on the basis of important interspecies differences, including differences in lipid metabolism (particularly germane in this study, in which CyA was shown to affect adversely plasma lipids, whereas rats are generally more resistant to atherogenic stimuli), prostaglandin metabolism, histopathologic aspects of the GCA lesions (*vide infra*), as well as method assessment of graft vessel disease (semi-quantitative earlier *versus* quantitative in this study). Importantly the more uniform lesions seen in this model with invariably intact IEL were not only more similar to human lesions (compared to the rat model) but also lent themselves to digitization and precise quantitative assessment. The dose response to fish oil has been investigated previously in hypercholesterol NZW rabbits (0.3% cholesterol diet)[16]: medium- and high-dose fish oil (approximately 200 and 300 mg n-3 fatty acids/kg/day, respectively) attenuated the development of native aortic and pulmonary atherosclerosis. The possible im-

TABLE 4. *Summary of Plasma Total Cholesterol,*

	Cholesterol (mg/dL)			Triglyceride (mg/dL)			VLDL (mg/dL)			LDL (mg/dL)		
Group	Baseline	At Death	p*	Baseline	At Death	p*	Baseline	At Death	p*	Baseline	At Death	p*
Control	35.0 ± 4.9	73.8 ± 9.3	<0.001	44.5 ± 7.0	130.0 ± 20.5	<0.007	8.8 ± 1.4	26.0 ± 4.0	<0.006	9.9 ± 2.8	34.1 ± 8.0	<0.006
SO(L)	55.1 ± 4.8	58.3 ± 7.1	NS	48.3 ± 5.3	69.3 ± 17.6	NS	9.5 ± 1.1	14.0 ± 3.5	NS	19.5 ± 4.0	20.3 ± 5.6	NS
SO(m)	50.2 ± 6.8	48.9 ± 6.1	NS	53.5 ± 12.7	54.0 ± 8.6	NS	10.7 ± 1.5	10.9 ± 1.7	NS	10.9 ± 5.1	15.0 ± 4.9	NS
SO(h)	57.0 ± 3.8	60.3 ± 6.0	NS	54.3 ± 8.6	65.7 ± 13.4	NS	10.9 ± 1.7	13.2 ± 2.7	NS	15.8 ± 4.0	17.0 ± 4.8	NS
FO(L)	53.8 ± 8.0	88.3 ± 9.3	<0.02	68.1 ± 10.4	65.2 ± 11.3	NS	13.7 ± 2.1	13.1 ± 2.2	NS	13.4 ± 7.0	46.9 ± 9.1	<0.02
FO(m)	48.5 ± 3.6	81.0 ± 10.2	<0.01	70.1 ± 13.1	71.6 ± 12.6	NS	14.0 ± 1.6	14.4 ± 2.5	NS	7.4 ± 2.4	42.1 ± 9.4	<0.003
FO(h)	51.4 ± 4.5	54.5 ± 5.2	NS	48.0 ± 4.4	64.2 ± 7.1	0.06	9.6 ± 0.9	12.8 ± 1.5	0.07	13.1 ± 3.9	17.8 ± 4.7	NS

* Differences within groups with respect to time (baseline *vs.* sacrifice), Student's t test for paired observations.

Lipid values are expressed as mean ± 1 SEM.
NS, not significant.

munomodulatory effects of fish oil between the two species in this study may account for the different results.

Rejection

Previous attempts to identify GCA-associated risk factors have been fairly disappointing. While an early study suggested an association with HLA-A_2 incompatibility and hypertriglyceridemia,[40] more recent follow-up demonstrated no consistent relationship with the number of rejection episodes during first post-transplant year, number of HLA mismatches, level of maintenance steroids, fasting blood sugar, or lipid profile,[1] although there was a weak correlation with older donor age. Despite the fact that most,[1,3,7] but not all,[2] studies did not show a significant correlation between GCA and extent of tissue mismatch or number of rejection episodes, most evidence implicates an immune-mediated phenomenon, possibly exacerbated by an atherogenic environment.[2,41,42] In this study the degree of rejection increased at higher oil doses, paralleling the graft vessel disease observations. Although not terribly strong, a direct correlation was apparent between all three parameters of GCA and RS (*vide supra*). This and the fact that recipient hearts had no observable lesions lend support to the hypothesis that GCA is indeed an immune-mediated phenomenon.

CyA and Lipids

CyA, a cyclic undecapeptide of fungal origin, is associated with fewer complications than conventional immunosuppressive therapy, such as corticosteroids and cytotoxic drugs, because of its specific action on T lymphocytes. It is, however, not without significant side effects. Notably chronic CyA administration produces hypertension and progressive renal insufficiency. Furthermore there have been many reports of possible CyA-associated elevations in TC, LDL, and TG[43–45]; however confounding factors included the concomitant use of steroids and/or other immunosuppressive agents. Our data indicate that CyA alone increased both TC and TG due to elevations of LDL and VLDL, respectively. No significant changes were noted in HDL and its subclasses. Similarly, in a previous study, rats given 30 mg/kg/day of CyA had significantly elevated TC and TG after 28 days,[46] which was prevented by fish oil; however the exact dose of omega-3 fatty acids was not specified. To date the exact mechanism by which CyA elevates lipid levels has not been elucidated. One poorly recognized adverse side effect of CyA is hepatotoxicity,[47] manifested primarily by impaired LDL hepatic clearance.[44] Thus lipid elevation with CyA may be related to altered hepatic lipoprotein metabolism.

In this study, while all doses of fish oil reduced TG and VLDL, only high-dose fish oil had an effect against CyA-induced elevations in TC and LDL. Because of a concomitant decrease in HDL (HDL_3) at this dose, fish oil did not significantly decrease the TC/HDL ratio, which has been associated with an increased risk of coronary artery disease.[48] In contrast safflower oil mitigated these adverse plasma lipoprotein changes at all doses. It may be that the P/S ratio (higher in safflower oil) is a stronger determinant of the hypolipidemic effect than the degree of unsaturation (higher double bond index in fish oil). The relative importance of the position of the first double bond, however, remains unclear.

Despite salutary effects on CyA-induced elevations in TC and LDL by safflower oil and high-dose fish oil (both also mitigated the increase in TG and VLDL), neither significantly reduced GCA in this model. The extent of GCA was similar in groups fed isocaloric amounts of fish oil and safflower oil. It appeared that the relative hyperlipidemic effect of CyA did not play a very important role in the pathogenesis of GCA in these rabbit allografts. In addition the lowering of LDL in itself may not be completely benign. Cyclosporine A is a lipophilic compound that partitions into phospholipid vesicles,[49] with more than 60% being transported in plasma among the various lipoprotein subclasses[50] in a nonsaturable manner below the therapeutic limit. Although not determined in the current study, reductions in plasma lipids, particularly TC, have been shown to decrease plasma and whole-blood CyA level,[46] which may actually increase the relative free CyA level thereby predisposing to CyA toxicity, particu-

Triglyceride, and Lipoprotein Subclasses

HDL (mg/dL)			HDL_2 (mg/dL)			HDL_3 (mg/dL)			Cholesterol/HDL		
Baseline	At Death	p*	Baseline	At Death	p*	Baseline	At Death	p*	Baseline	At Death	p*
17.2 ± 3.1	13.6 ± 2.3	NS	3.6 ± 1.8	1.7 ± 0.8	NS	14.5 ± 1.7	11.9 ± 2.0	NS	2.1 ± 0.1	6.4 ± 1.0	0.002
27.1 ± 1.5	24.2 ± 2.2	NS	1.6 ± 0.9	0.8 ± 0.7	NS	25.8 ± 1.2	24.3 ± 1.6	NS	2.1 ± 0.2	3.0 ± 0.8	NS
29.5 ± 2.1	23.0 ± 1.5	<0.04	3.7 ± 0.9	1.4 ± 0.8	<0.03	25.8 ± 1.6	21.6 ± 1.2	NS	1.7 ± 0.2	2.2 ± 0.4	NS
31.0 ± 2.4	30.2 ± 2.6	NS	4.2 ± 1.5	2.9 ± 1.5	NS	26.8 ± 2.0	27.3 ± 2.1	NS	2.0 ± 0.2	2.3 ± 0.4	NS
29.9 ± 2.3	28.6 ± 2.8	NS	5.4 ± 1.1	2.4 ± 1.6	NS	24.4 ± 1.8	26.1 ± 2.3	NS	1.8 ± 0.3	3.5 ± 0.5	<0.03
28.2 ± 2.8	24.5 ± 2.4	NS	2.8 ± 1.1	1.8 ± 1.4	NS	25.4 ± 2.1	22.7 ± 1.6	NS	1.9 ± 0.3	3.7 ± 0.7	<0.005
29.3 ± 2.0	23.1 ± 1.4	<0.002	1.8 ± 0.7	1.1 ± 0.9	NS	27.6 ± 1.9	22.1 ± 1.2	<0.004	1.8 ± 0.2	2.5 ± 0.3	NS

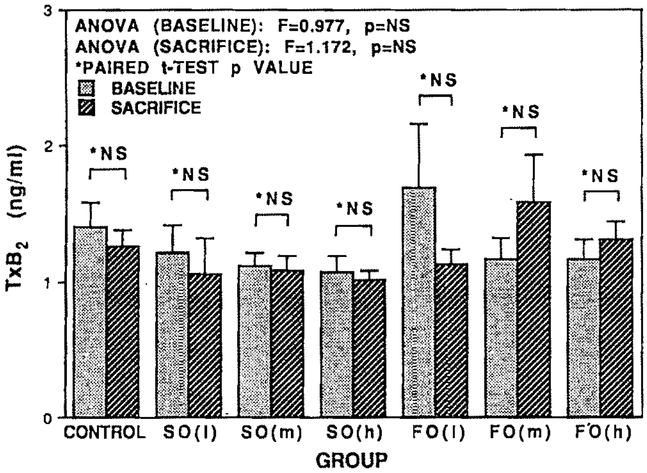

FIG. 5. Levels of serum thromboxane B_2 (TxB_2) for all groups at baseline and at death. Neither fish oil nor safflower oil at any dose affected serum TxB_2. ANOVA = analysis of variance. Values are expressed as mean ± 1 SEM.

larly the central nervous system.[51] Such effects also may lead to endothelial cell (EC) injury,[52] increased platelet activity,[53,54] and inhibition of prostacyclin production,[55] all of which are possible risk factors favoring accelerated GCA.

Serum Thromboxane

Omega-3 fatty acids are known to inhibit TxA_2 formation[56,57] while producing a small amount of physiologically inactive TxA_3.[56,58] Although a poor substrate, EPA avidly binds to platelet cyclooxgenase and competes with arachidonic acid,[59] resulting in reduced TxA_2 production. Serum TxB_2 was unaffected by all doses of fish oil in this study, which is in disagreement with a previous investigation of cholesterol-fed rabbits supplemented with fish oil.[16] In that study TxB_2 was reduced in the control group and baseline values also were much higher. Perhaps basal levels of serum TxB_2 in this current study were already so low that it would be difficult to detect a further decrease given the sensitivity of the radioimmunoassay used. Furthermore, there are associated pitfalls[60] with measurement of plasma and serum TxB_2 by radioimmunoassay (RIA); methodology in the preparation of blood samples and RIA may have accounted for the discrepancy between the two studies. It has also been suggested that CyA may increase platelet aggregation and TxB_2 generation,[53,54] which may counteract any possible TxB_2-lowering effect of omega-3 fatty acids. To ascribe any beneficial effects of fish oil on arteriosclerosis to TxA_2 production alone, however, would be overly simplistic. No significant correlation was found between GCA (as measured by $\%LO_m$, $\%LO_c$, or IT) and either serum TxB_2 or percentage change in TxB_2 in this study. This is supported by Zhu et al.,[16] who also found no correlation between changes of TxB_2 and percentage of lesions in the aorta or pulmonary artery (r = 0.128).

LDL Oxidation Potential

Recently much attention has been directed to the concept that oxidatively modified LDL may play a major role in the development of atherosclerosis.[61] *In vitro* LDL incubated with monocytes/macrophages,[62,63] ECs,[39,64,65] and smooth muscle cells[65,66] is converted to a modified form that is avidly degraded by macrophages *via* scavenger receptors leading to accumulation of cholesterol esters and foam cell formation. Modified LDL also exhibits chemotactic activity for human monocytes but inhibits the chemotactic response of mouse peritoneal macrophages to EC-derived chemotactic factors.[67] Furthermore lipid peroxidation products and modified LDL particles are themselves cytotoxic.[68] The recent demonstration of autoantibodies against MDA-LDL in rabbit and human sera[69] as well as isolation of oxidatively modified forms of LDL from human plasma[70] and atherosclerotic lesions[63,71] has lent support to the importance of *in vivo* oxidation of LDL. It is apparent from recent studies that biologically oxidized LDL also may play an important role in retarding arteriosclerosis. Chemically modified LDL, *in vitro*, caused a marked suppression of platelet-derived growth factorlike factor (PDGF-c) production by cultured bovine aortic and human umbilical vein ECs.[72] Furthermore fish oil extract (30% polyunsaturated n-3 fatty acids) inhibited EC production of PDGF-c by 80% to 90%, while safflower oil (74% linoleic acid; C18:2n-6) was 5% to 10% as potent and peanut oil (primarily satu-

TABLE 5. *LDL Lipid Peroxidation Level With and Without Copper Oxidation as measured by Thiobarbituric Acid Reactive Substance (TBARS)*

	TBARS (nmol MDA equivalent/mg LDL protein)	
Group	No CU^{++}	CU^{++}
Control	6.7 ± 0.7	47.8 ± 1.1*
SO(l)	9.2 ± 2.1	52.3 ± 3.9*
SO(m)	6.1 ± 1.9	61.4 ± 6.2*
SO(h)	2.3 ± 1.1	45.2 ± 5.6*
FO(l)	25.9 ± 1.9†	90.7 ± 4.5‡
FO(m)	11.7 ± 6.4	99.3 ± 7.6‡
FO(h)	29.3 ± 7.4†	152.3 ± 11.0
ANOVA§	p = 0.0001	p = 0.0001

* $p < 0.05$ *vs.* FO(l), FO(m), and FO(h).
† $p < 0.05$ *vs.* Control, SO(l), SO(m), SO(h), and FO(m).
‡ $p < 0.05$ *vs.* FO(h).
MDA = malondialdehyde.
§ ANOVA p values correspond to differences between groups.
Values are expressed as mean ± 1 SEM.

rated and monounsaturated fatty acids) had only a negligible effect[73]; this suggested a possible correlation with the degree of fatty acid polyunsaturation. It was proposed that the inhibition of EC-derived mitogens by fish oil may inhibit myointimal proliferation and arteriosclerotic plaque formation.[73]

In this study baseline LDL oxidation level was significantly higher with low and high fish oil doses. With Cu^{++} oxidation levels were significantly elevated in all fish oil groups, reflecting that the high polyunsaturation of n-3 fatty acids leads to greater lipid peroxidation. A dose response to fish oil was noted: Cu^{++} oxidation potential was not significantly higher in FO(m) than FO(l), perhaps reflecting the low baseline lipid peroxidation level of the FO(m) group. This trend favored greater GCA, however, with increasing fish oil dose. Perhaps, at increasing oxidation potential of LDL, the beneficial effect of inhibiting EC mitogen production may be overcome by the cytotoxic and atherogenic (foam cell formation) properties of oxidized LDL. On the other hand, although all safflower groups had comparable oxidation potential to control animals, a significantly higher amount of GCA also was seen with high-dose safflower oil. It may be that LDL oxidation potential may not reflect the actual degree of lipid peroxidation in the local milieu of arteriosclerotic plaques, where most of the LDL modification must occur because plasma contains effective endogenous antioxidants, in particular, ascorbate and albumin-bound bilirubin.[74]

Limitations

Several potential limitations of this experiment should be addressed. Whether nonfunctional heterotopic cardiac allografts are different from orthotopically placed hearts in terms of GCA and their response to dietary manipulations remains unknown. Given the major technical difficulties in performing orthotopic cardiac transplants in rabbits and the relative rapid development of graft vessel disease (which is remarkably similar to human GCA and easily quantified) in these heterotopic allografts, we think that this may be a comparable, if not superior, small-animal model of human GCA compared to those previously used.

The number of experimental groups ($n = 7$) and measurements at two different times (baseline *versus* time of death) mandated the use of rigorous, conservative methods of statistical analysis (ANOVA with appropriate multiple comparison tests and Bonferroni corrections, and repeated measures ANOVA). Because we initially intended to use the same control group for comparison to other concurrent drug treatment groups, we chose to be even more conservative in terms of p values considered to be statistically significant. Therefore, although we tried not to ignore trends in the data, it is possible that we failed to detect small, but potentially important, differences (type II statistical error).

Some differences, albeit minimal, do exist in the histopathology of graft coronary arterial lesions seen in this model and human GCA. Due to interspecies differences in biologic systems, extrapolation of the results in this study to humans must be made with caution. There are obvious differences between rabbit and human lipid metabolism, as were reflected in the lower levels of TG, TC, VLDL, and LDL in rabbits and their responses to dietary fat manipulation (*vida supra*). Although we did not assess the dose response of n-3 fatty acid incorporation, this was measured indirectly by the extent of LDL lipid peroxidation by copper. It is possible that LDL modification at the level of the local milieu may not be reflected by *in vitro* LDL oxidation potential by transition metals as measured by TBARS. This can be potentially circumvented in future studies by performing quantitative analyses, using more sensitive methods, such as mass spectrophotometry/high-performance liquid chromatography, for fatty acid and lipid hydroperoxide composition of arteriosclerotic plaque extracts.

Finally recent experimental data has redirected attention to the emerging roles of cytokines in the pathogenesis of arteriosclerosis. Omega-3 fatty acids have been demonstrated to reduce the synthesis of interleukin-1 (IL-1) and tumor necrosis factor[75] by mononuclear cells, an effect lasting for as long as 10 weeks after cessation of n-3 fatty acid supplementation. Furthermore EPA, a favored substrate over arachidonic acid in the lipooxygenase pathway, also inhibits the production of LTB_4,[76] *in vitro*, *via* an inhibitory effect of LTA_3 on leukotriene A hydrolase,[77] which normally converts LTA_4 to LTB_4. Omega-3 fatty acids also have been shown to reduce production of PGE_2,[78] which usually acts as a feedback inhibitor of cellular immune responses such as T-cell proliferation, lymphokine production, and cytotoxicity.[79] Prostaglandin E_2 also suppresses MHC class II antigen expression.[80] This would be expected to counteract any n-3 fatty acid-mediated suppression of monokine release. In contrast to n-3 fatty acids, n-6 fatty acids also reduced IL-1 levels in rat Kupffer cells, after 6-weeks of safflower oil dietary supplementation, but without affecting PGE_2 release.[78] Although these processes constitute undoubtedly relevant, if not more important, factors to be considered in this immune-mediated accelerated cardiac GCA model, they were not directly investigated in the present study. In addition the extent of MHC class I and II mismatches, CyA dose and route of administration, and dose, composition, and formulation of oil preparation may be important variables to be considered when interpreting the observed data.

In summary GCA in this rabbit heterotopic cardiac transplant model closely resembled human GCA and appeared to be affected in an unfavorable manner by fish oil and safflower oil administration, independent of their effects on plasma lipids and LDL oxidation potential. The data do not support the potential usefulness of fish oil or safflower oil to control GCA, although safflower oil may be beneficial at low doses. The metabolic and immunomodulatory effects of both types of dietary fat supplementation are numerous and multifactorial. It is probable that the degree of GCA ultimately will depend on a combination of effects exerted by each fatty acid (n-3 *versus* n-6), including those not investigated in this study. Further studies, animal and clinical, are needed to determine the utility of polyunsaturated fatty acid supplementation (whether it be fish oil, safflower oil, or other fats) in the prevention of accelerated human cardiac GCA and to elucidate further their mechanisms of action.

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Adverse Effect of Therapeutic Vasoconstrictors in Experimental Acute Pancreatitis

E. KLAR, M.D.,* D. W. RATTNER, M.D.,† C. COMPTON, M.D.,‡ G. STANFORD, M.D.,§
B. CHERNOW, M.D.,§ and A. L. WARSHAW, M.D.†

Alpha-adrenergic drugs commonly are used to treat hypotension resulting from severe acute pancreatitis. It was shown previously that although systemic arterial pressure is increased by phenylephrine, pancreatic microcirculatory perfusion is decreased. Because inadequate tissue perfusion may be critical in the progression of edematous pancreatitis to parenchymal necrosis, it was hypothesized that vasoconstrictors might be harmful in pancreatitis. Therefore the effect of phenylephrine on cerulein-induced mild pancreatitis were studied. Sprague-Dawley rats (n = 54) were randomly allocated to 6 experimental groups and subjected to the following infusion regimens: (1) cerulein (cae) + phenylephrine (phe), (2) cae + saline (NS), (3) NS + phe, (4) cae + phenoxybenzamine (pbz) + phe, (5) NS + pbz + phe, and (6) NS. Initial and terminal hematrocrit, serum amylase activity, and blood ionized calcium concentration were determined. The animals were killed 9 hours after starting the infusion. Macroscopic and histologic changes were scored by a 'blinded' pathologist. Phenylephrine increased the severity of cerulein-induced pancreatitis as manifested by statistically significant adverse changes in serum amylase, hematocrit, ionized calcium, peripancreatitic soap formation, and acinar cell vacuolization. These changes were antagonized by alpha-adrenergic receptor blockade with phenoxybenzamine. It is concluded that phenylephrine is deleterious in acute experimental pancreatitis, the first demonstration of such an effect by a pharmacologic vasoconstrictor, and suggested that microcirculatory changes may be important in the transition of mild to severe pancreatitis. Caution in the use of vasoconstrictor drugs in patients with acute pancreatitis is recommended.

From the Department of General Surgery, University of Heidelberg, Heidelberg, West Germany; and the Departments of Surgery,† Pathology,‡ and Anesthesia,§ Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts*

WHILE THE TRADITIONAL concept of acute pancreatitis is that the primary injury is enzymatic, there is increasing evidence that ischemia may be critical in the initiation of or progression to necrosis.[1] Experimental[2-5] and clinical[6-8] studies have indicated that the pancreas is highly susceptible to ischemic damage. Dynamic contrast-enhanced computed tomographic (CT) scans both in humans and in experimental animals have shown that reduced perfusion of the microcirculation early in the course of acute pancreatitis correlates with more severe pancreatitis and in particular with the development of areas of necrosis.[9] Variations in pancreatic perfusion therefore may alter the course of the illness.

Alpha-adrenergic vasoconstricting drugs commonly are used as an adjunct in treating the hypotension characteristic of severe early acute pancreatitis. However we have observed that phenylephrine, an alpha-1 receptor agonist, markedly reduces pancreatic microcirculatory perfusion in the rat[10] and have also noted an association between the increasing use of vasoconstrictors and pancreatic injury in patients undergoing cardiopulmonary bypass.[8] Therefore we investigated the effect of phenylephrine on the severity of cerulein-induced pancreatitis in rats.

Materials and Methods

The following studies were approved by the Subcommittee on Animal Care at the Massachusetts General Hospital.

Male Sprague-Dawley rats (Charles River Labs, Chelmsford, MA), weighing 320 to 350 g, were anesthe-

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Address reprint requests to Dr. E. Klar, Chirurg. Univ. Klinik, Im Neuenheimer Feld 110, 69 Heidelberg, Germany.

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tized for vascular cannulation by administering intraperitoneal ketamine (44 mg/kg) and pentobarbital (20 mg/kg). Using aseptic technique a PE-10 polyethylene catheter (Becton-Dickinson, Parsipanny, NJ) was inserted into the right internal jugular vein for infusion of drugs. An additional PE-10 catheter was placed into the right carotid artery for blood sampling and arterial pressure monitoring. Both catheters then were tunnelled subcutaneously to the subscapular area and brought out through a lightweight flexible spring. The catheters were flushed with 0.5 mL of heparinized saline (10 U/mL) after insertion. The arterial catheter was subsequently flushed with 1 mL heparinized saline 6 and 18 hours after the operation. The animals were housed in individual stainless steel cages and allowed to recover overnight. The spring containing the catheters exited the cage through a hole on top and was connected to a swivel that permitted unrestrained movement of the animal. The animals were given water *ad libitum*. The experiments were performed on unanesthetized conscious animals in their home cages the next day.

Arterial pressure was monitored using a Spectramed (072911-00-075, Spectramed, Oxnard, CA) transducer linked to an 8-channel Hewlett-Packard Recorder 7758A (Hewlett-Packard, Downers Grove, IL). Mean arterial pressure (MAP) and pulse were recorded for 1-minute segments at each time point described below. Blood samples were collected through the arterial catheter and analyzed for serum amylase activity,[11] blood ionized calcium (ion-selective electrode "Nova 2," Nova Biomedical, Waltham, MA), and hematocrit.

In all animals 0.05 mL of blood was withdrawn for measuring baseline hematocrit and replaced by 0.2 mL of 0.9% NaCl 30 minutes before the assessment of baseline blood pressure and heart rate. Heart rate, pulse, and MAP were recorded while rats were resting quietly, starting approximately 18 hours after placement of catheters. The hemodynamic parameters were followed hourly as well as 10 minutes after starting the phenylephrine infusion and 10 minutes after administration of phenoxybenzamine, respectively. Throughout the experimental period, the arterial catheter was connected to a high-pressure infusion system delivering 0.9 mL per hour of heparinized saline (1 unit per mL). Nine hours after the start of the infusion, blood was withdrawn from the arterial catheter for determination of hematocrit, serum amylase activity, and ionized calcium concentration. The animals were killed by a lethal injection of intravenous pentobarbital.

Experimental Design

Fifty-four rats were distributed randomly to six experimental groups (Table 1). To study the effects of vasoconstriction, 30 rats were allocated to experimental groups I to III. To investigate the effects of alpha receptor blockade, an additional 24 rats were allocated randomly to experimental groups IV to VI. The treatment groups are shown schematically in Figure 1 and described below.

TABLE 1. *Treatment Groups*

Group	Treatment
I	Cerulein + phenylephrine
II	Cerulein + normal saline
III	Normal saline + phenylephrine
IV	Cerulein + phenoxybenzamine + phenylephrine
V	Normal saline + phenoxybenzamine + phenylephrine
VI	Normal saline

Group I (cerulein + phenylephrine, n = 12): Acute edematous pancreatitis was induced by the intravenous bolus injection of 3 μg/kg of cerulein (Peninsula Labs., Belmont, CA) followed by a continuous infusion of 5 μg/kg/hr (0.48 mL/hr) for 6 hours. Four hours after commencing the cerulein infusion, phenylephrine HCL (neosynephrine, Winthrop-Brenon, New York, NY), diluted in NS, was infused at a rate of 3 mg/kg/hr, allowing for a constant total infusion volume of 0.94 mL per hour. The infusion was terminated 2 hours after completing the cerulein infusion (total of 4 hours of phenylephrine infusion). Animals were killed 1 hour after completing the phenylephrine infusion (*i.e.*, 9 hours after beginning the cerulein infusion).

Group II (cerulein + normal saline, n = 10): Acute edematous pancreatitis was induced as in group I. Four hours after commencing cerulein infusion, normal saline was infused for 4 hours at a rate of 0.94 mL/hr.

Group III (normal saline + phenylephrine, n = 8): Rats were given a 0.3-mL bolus of normal saline at the start of the experiment followed by an infusion of 0.48 mL/hr. Four hours after commencing the saline infusion, phenylephrine HCL was infused at a rate of 50 μg/kg/min (0.94 mL/hr) for 4 hours.

Group IV (cerulein + phenoxybenzamine + phenylephrine, n = 7): Cerulein infusion was begun as in group I. Three hours and 15 minutes after beginning cerulein infusion, 5 mg of phenoxybenzamine (5 mg/mL) in normal saline was infused over 15 minutes to establish alpha blockade. Four hours after beginning the cerulein infusion, phenylephrine infusion was begun in a manner identical to that of groups I and III.

Group V (normal saline + phenoxybenzamine + phenylephrine, n = 8): Animals were given saline infusion rather than cerulein. Phenoxybenzamine and phenylephrine were administered exactly as in Group IV.

Group VI (Normal saline controls, n = 7): Appropriate volumes of normal saline were used in identical volume

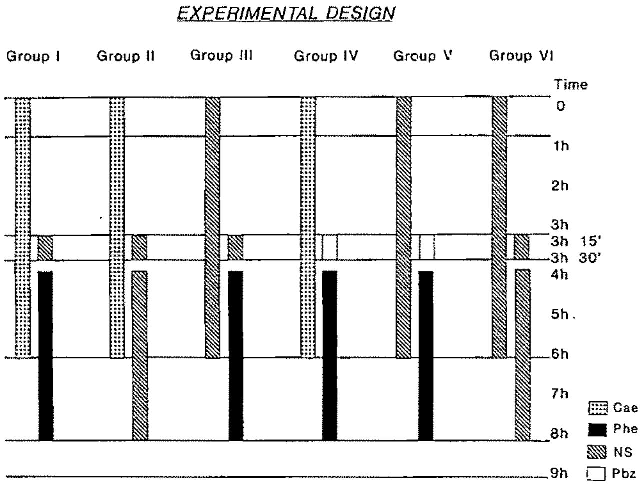

FIG. 1. Schematic diagram showing the sequence and combinations in which cerulein(cae), phenylephrine(phe), and saline(ns) were administered to the experimental group.

and time sequence as substitutes for cerulein, phenoxybenzamine, and phenylephrine following the sequence of Group IV.

Morphology

After the animals were killed, postmortem examination of the abdomen was performed by a 'blinded' pathologist. Pancreatic edema and peripancreatic soap formation were graded according to the following scale: Pancreatic edema, + = confined to the tail, maximal the width of the spleen, ++ = head and tail, +++ = head and tail, the latter exceeding the width of the spleen; peripancreatic soap formation, + = 1 focus, ++ = 2 foci, +++ = confluent areas.

Thereafter the whole pancreas (with duodenum and spleen attached) was removed, fixed in 10% buffered formalin, and embedded in paraffin. Six histopathologic parameters were analyzed quantitatively from a single transverse section through the long axis of the pancreas: *i.e.*, edema, parenchymal necrosis, inflammation, vacuolization, hemorrhage, and fat necrosis. Edema was scored as follows: 1+ if interlobular septae were expanded; 2+ if intralobular septae were expanded; and 3+ if individual acini were separated by edema. Parenchymal necrosis was quantified as follows: 1+ = 1 to 4 necrotic acinar cells per high-power field (HPF); 2+ = 5 to 10 cells per HPF; 3+ = confluent aggregates of more than 10 necrotic acinar cells. Inflammation was scored as follows: 1+ = 2 to 10 intralobular neutrophils per HPF; 2+ = more than 10 intralobular neutrophils per HPF without confluent aggregates or microabscesses; 3+ = confluent aggregates or microabscesses. Vacuolization was scored as 1+ if less than one third of the acinar cells in a HPF had prominent cytoplasmic vacuoles; 2+ if between one third and two thirds of the acinar cells had vacuoles; and 3+ if more than two thirds had vacuoles. Hemorrhage and fat necrosis were scored as follows: 1+ = 1 focus per slide; 2+ = 2 foci per slide; and 3+ = 3 or more foci per slide.

Statistical Analysis

All values are expressed as mean ± standard deviation (SD). All parameters were tested for normal distribution. The following data were assessed by Student's t test: MAP, heart rate, and blood-ionized calcium. Because of large variance, especially in group I, serum amylase and hematocrit were subjected to Fisher's exact test. Probability values less than 0.05 were considered significant.

Results

Serum Amylase

As expected cerulein infusion caused a significant increase in the serum amylase activity (groups I, II, and IV) when compared with each of the experimental groups not receiving cerulein (Fig. 2). The addition of phenylephrine to cerulein caused a significantly higher amylase concentration than cerulein infusion alone (group I, 1076 ± 647 U/L *versus* group II, 709 ± 128 U/L ($p < 0.05$).

Blood-ionized Calcium

Blood-ionized calcium was significantly lower in group I (cae + phe) than group II (cae + ns) ($p < 0.05$) (Fig. 3). The difference between group I and group IV approximated significance ($p = 0.05$), suggesting that phenoxybenzamine partially counteractivated the effect of phenylephrine.

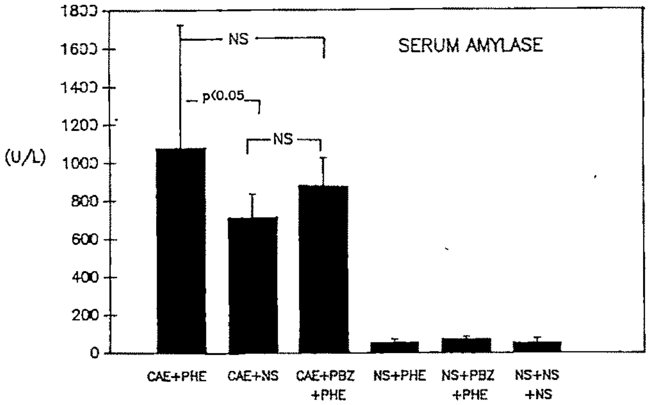

FIG. 2. Serum amylase activity in animals receiving cerulein plus phenylephrine infusion was significantly higher than animals receiving cerulein and normal saline infusion. Phenylephrine infusion alone did not alter control levels of serum amylase.

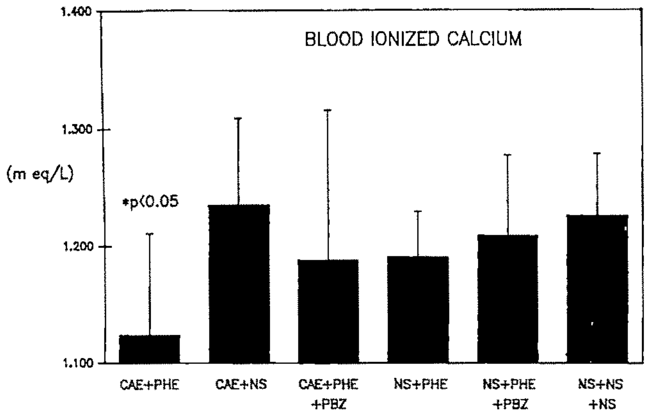

FIG. 3. Blood ionized calcium was significantly lower in rats receiving cerulein and phenylephrine that rats receiving cerulein + normal saline.

Hematocrit

The mean hematocrit level in group I increased by 4.75% ± 13.7% in contrast to all other groups, which showed decreases in the hematocrit (Fig. 4). This difference was statistically significant ($p < 0.05$ by Fisher's exact test). There was no significant difference between group II and group IV. Thus phenylephrine superimposed changes on cerulein-induced pancreatitis that were abolished by phenoxybenzamine.

Gross Pathology

Significantly more peripancreatic fat necrosis was observed in group I (cae + phe) than in groups II (cae alone) or IV (cae + pbz + phe) (Table 2). This observation is again consistent with an adverse effect of phenylephrine on cerulein pancreatitis and blockade of the effect by phenoxybenzamine. There were no other significant differences in the macroscopic appearances among the groups with acute pancreatitis. The pancreas appeared normal on gross inspection in the control groups (III, V, and VI).

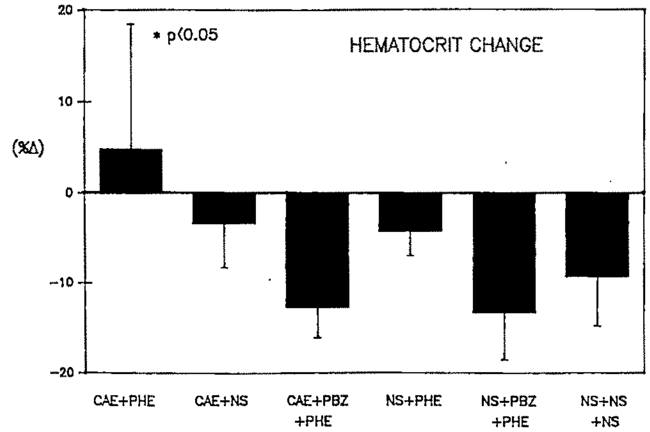

FIG. 4. Hemoconcentration, manifested as an increase in the mean hematocrit, occurred only in animals receiving cerulein and phenylephrine infusion.

TABLE 2. *Gross Pathology*

Group	Edema	Peripancreatic Fat Necrosis
I	12/0	4/8*
II	10/2	0/12
III	1/7	0/8
IV	6/1	0/7
V	0/8	0/8
VI	0/8	0/8

* = $p < 0.01$ *vs.* all other groups.

Data are expressed as a ratio: number of rats with changes > 1+/number of rats with changes ≤ 1+. Peripancreatic soap formation was greatest in animals receiving cerulein and phenylephrine.

Histology

Pancreatitis was seen only in animals that received cerulein infusion (groups I,II, and IV) (Table 3). The extent of edema, inflammation, hemorrhage, and necrosis did not significantly differ among the groups with acute pancreatitis. Microvascular thrombosis and vasculitis were not frequently observed. Significantly more vacuolization was seen in rats receiving cerulein plus phenylephrine (group I) compared to those receiving cerulein alone (group II). This change was not abolished by alpha receptor blockade (group IV). In three rats the quality of the histologic preparation was not suitable for analysis.

Hemodynamics

Phenylephrine infusion (50 μg/kg/min) increased MAP from 110 ± 11 mmHg to 144 ± 10 mmHg (Fig. 5A). There was a concurrent decrease in heart rate (423 ± 39 to 336 ± 57 beats/min (Figure 6A). Mean arterial pressure promptly returned to baseline with cessation of phenylephrine infusion. Phenoxybenzamine infusion caused a

TABLE 3. *Histology*

Group	Edema	Inflammation	Necrosis	Vacuolization
I	12/0	9/3	8/4	12/0*
II	10/0	7/3	4/6	4/6
III	3/5	0/8	0/8	0/8
IV	7/0	6/1	2/5	7/0*
V	0/8	0/8	0/8	0/8
VI	0/7	0/7	0/7	0/7

* = $p < 0.05$ *vs.* group II.

Data are expressed as a ratio: number of rats with changes > 1+/number of rats with changes ≤ 1+. Cerulein and phenylephrine produced significantly more vacuolization than cerulein alone. This change was not abolished by phenoxybenzamine.

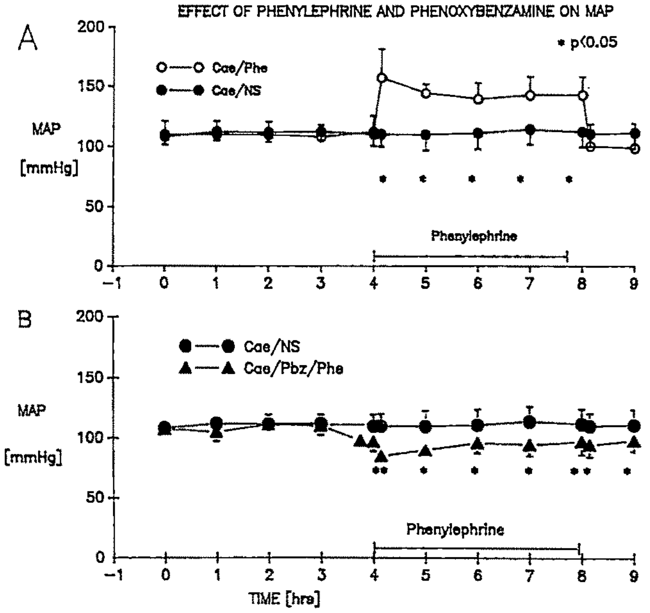

FIG. 5. Intravenous infusion of phenylephrine caused an immediate and significant increase in MAP (A). Alpha-adrenergic blockade by phenoxybenzamine caused a decrease in MAP. The addition of phenylephrine in the presence of alpha blockade caused a further decrease in MAP due to unmasking the weak beta agonist activity of phenylephrine (B).

decrease in MAP from 110 ± 7 mmHg to 97 ± mmHg ($p < 0.05$). The addition of phenylephrine in the presence of established alpha blockade by phenoxybenzamine led to a further decrease in MAP (Fig. 5B). Accompanying the decrease in blood pressure was an increase in heart rate (Fig. 6B).

Mortality

One rat in group IV died shortly after beginning the intravenous infusion, presumably due to catheter-related embolism. No other deaths occurred during the experimental period.

Discussion

The importance of reduced blood flow in the pathogenesis of acute pancreatitis has been investigated in a variety of ways. In 1948 Popper[2] demonstrated that short-term arterial occlusion following the induction of edematous pancreatitis (by duct ligation and hyperstimulation) led to the development of necrosis. Pfeffer's[3] work focused attention on the importance of the microcirculation. He demonstrated that obstruction of terminal arterioles by the intra-arterial injection of 8 to 20 μ microspheres caused hemorrhagic pancreatitis. In contrast larger microspheres produced only pancreatic edema, presumably because collateral flow was capable of maintaining microcirculatory perfusion. Subsequent investigators have confirmed that microcirculatory disturbances occur in the pathogenesis of experimental pancreatitis.[12-17] Intravital microscopy has demonstrated directly capillary blood flow impairment within 60 minutes of the induction of biliary pancreatitis.[18]

Clinical evidence also supports the importance of ischemia in the pathogenesis of pancreatitis. In 1978 Warshaw and O'Hara[6] noted a high incidence of pancreatitis in cardiac surgical patients who were in a low output state. This relationship was confirmed in subsequent studies.[7,8] Recently contrast-enhanced CT has been used to study pancreatic perfusion and the subsequent development of pancreatic necrosis. The extent of hypoperfused pancreas on admission CT scans predicts the subsequent development of pancreatic necrosis, abscesses, and death.[9]

Phenylephrine is an alpha adrenergic agent widely used to produce vasoconstriction in critically ill patients. Phenylephrine increases total peripheral resistance and can be accompanied by considerable hemodynamic changes, including a decrease in cardiac output and reduced splanchnic blood flow.[19,20] In rats it causes increases in MAP and total peripheral resistance and a decrease in cardiac output at doses between 3 and 10 μg/kg/min.[19] Despite increased MAP, doses greater than 25 μg/kg/min

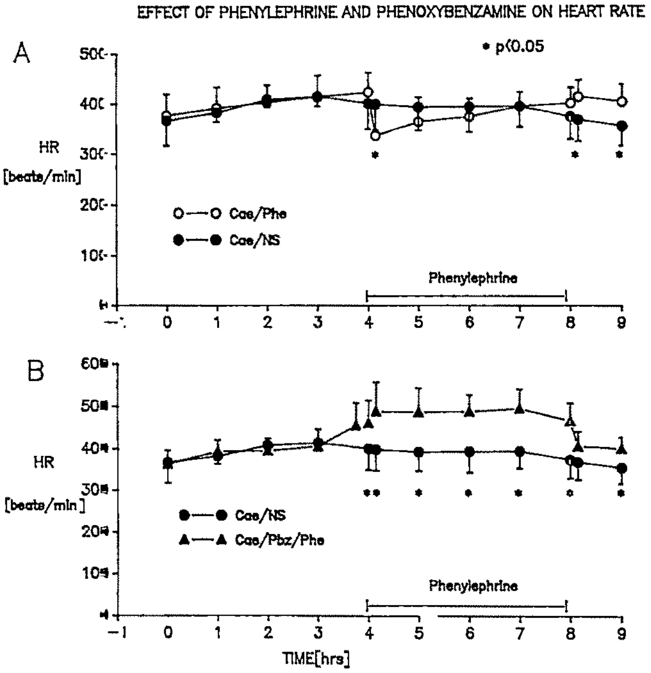

FIG. 6. Phenylephrine infusion initially caused bradycardia in conjunction with an increase in MAP when compared to animals receiving cerulein infusion alone. However, after completion of the infusion, animals that had received cerulein plus phenylephrine had a significantly increased tachycardia, presumably due to intravascular volume contraction (A). Phenylephrine infusion in the presence of alpha blockade by phenoxybenzamine accentuated tachycardia due to inherent weak beta agonist activity of phenylephrine.

decrease splanchnic perfusion.[21] This finding has been ascribed to vasoconstriction and has been demonstrated in experimental preparations as well as in humans.[20–25] We have shown with intravital microscopy in the rat that phenylephrine reduces pancreatic microcirculatory flow to 36% of controls.[10] Recently, using reflectance spectroscopy, we also have found that oxyhemoglobin levels in pancreatic tissue decrease by 25% and deoxyhemoglobin levels increase correspondingly during phenylephrine infusion.[26]

In the current study, a dose of 50 μg/kg/min was used to produce splanchnic vasoconstriction. This dose is well tolerated by the rat for up to 6 days.[27] In our model the hemodynamic effect of phenylephrine was documented by an increase in MAP of 30 to 40 mmHg accompanied by bradycardia[19,28] (Figs. 5 and 6). These changes were completely abolished with alpha receptor blockade by phenoxybenzamine as previously described.[29,30] The characteristic decrease in blood pressure and increase in heart rate were augmented by the subsequent addition of phenylephrine. This was due to the weak beta-agonist activity of phenylephrine.

In this study phenylephrine administration increased the severity of cerulein-induced pancreatitis. This finding was confirmed by higher serum amylase activity, hemoconcentration, hypocalcemia, an increase in pancreatic soap formation, and an increase in acinar cell vacuolization.

In certain experimental models, serum amylase activity correlates with the severity of pancreatitis (in contrast to most clinical situations). In Sokolowski's model using short-term ischemia of the pancreas, the serum amylase level correlated well with varying degrees of pancreatic injury.[4] In the cerulein model of pancreatitis, serum amylase levels parallel the histologic changes.[31–33] Therefore it seems appropriate, in our model, to interpret the significantly higher amylase in group I (cerulein + phenylephrine) as an indication of more severe pancreatitis.

Hemoconcentration is a common finding in patients presenting with acute pancreatitis and the volume of intravenous fluid necessary to reverse this change is generally accepted as a parameter reflecting the severity of the disease.[34] In our studies hemoconcentration was only observed in the animals that received cerulein and phenylephrine. Mild hemodilution was observed in all other groups. This fact provides further evidence of more severe pancreatitis in group I.

Hypocalcemia is also a well-established prognostic marker for the severity of pancreatitis.[34] Although many mechanisms have been proposed to account for hypocalcemia, no single explanation has been widely accepted. While it is tempting to attribute hypocalcemia to sequestration of calcium in areas of fat necrosis, human autopsy studies have shown that the quantity of calcium in soap formation is insufficient to account for the observed changes.[35,36] Nonetheless, in rats, the extent of intra-abdominal fat necrosis correlates with severity of pancreatitis.[4,5,37] In these experiments hypocalcemia was only observed in animals receiving both cerulein and phenylephrine. Rats receiving both cerulein and phenylephrine also had the most pronounced peripancreatic soap formation. These data provide further evidence that phenylephrine caused a worsening of cerulein-induced pancreatitis.

Morphologic changes of pancreatitis were only seen in animals receiving cerulein. The histologic changes of pancreatitis were similar to those described originally by Lampel and Kern[38] and Tani.[33] Phenylephrine alone or in combination with phenoxybenzamine did not cause pancreatitis. In the cerulein model of pancreatitis, vacuolization is a relatively early change, seen within the first 9 hours of the illness. In contrast inflammatory cell infiltration is more prominent at a slightly later time period (9 to 12 hours). Those animals receiving phenylephrine in addition to cerulein demonstrated more vacuole formation than other groups receiving cerulein alone or in combination with phenoxybenzamine. The 9-hour period of observation in our study was too brief to evaluate the full extent of inflammation and necrosis, but the increased vacuolization seen in group I is histologic evidence of more severe pancreatitis.

It seems most likely, in consideration of the known effects of phenylephrine on the pancreatic microcirculation, that the observed harmful effects of phenylephrine on otherwise mild cerulein-induced pancreatitis are due to impairment of pancreatic perfusion, rather than some other unspecified action on acinar cell membrane receptors. Despite increased MAP both cardiac output and splanchnic blood flow have been documented to decrease under similar experimental conditions.[21] Although we did not quantitate pancreatic perfusion in this study, the proposed mechanism is supported by the abolition of these changes by phenoxybenzamine. Despite a significant decrease in MAP in animals receiving phenoxybenzamine + phenylephrine + cerulein, most of the changes indicative of more severe disease were prevented. A previous study using an identical dose of phenoxybenzamine demonstrated that pancreatic perfusion was preserved in the presence of alpha blockade, despite a decrease in MAP of 32 mmHg.[39] Furthermore it has been shown that improvement of pancreatic perfusion during biliary pancreatitis reduces the extent of parenchymal injury.[40]

The present study demonstrates that mild interstitial pancreatitis can be worsened by the systemic administration of phenylephrine. While there is much previous evidence that ischemic injury may be an important factor in the aggravation and progression of acute pancreatitis, this is the first demonstration that a pharmacologic agent causing reduced blood flow can augment the injury. Whether this phenomenon is due to direct action on the pancreatic microcirculation or an indirect action *via* globally diminished splanchnic blood flow is not shown by these experiments. In either case the protection afforded by alpha adrenergic blockade supports the hypothesis that pancreatic hypoperfusion is the mechanism of action. These experimental results lead us to suggest that vasoconstrictor agents may worsen pre-existing pancreatitis in humans and therefore should be avoided if possible.

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The Local Effects of Cachectin/Tumor Necrosis Factor on Wound Healing

GARY D. SALOMON, M.D., ATTAN KASID, PH.D., DOUGLAS T. CROMACK, M.D., ELAINE DIRECTOR, B.S.,
THOMAS L. TALBOT, M.S., ANTHONY SANK, M.D., and JEFFREY A. NORTON, M.D.

Previous experimental studies have suggested that tumor necrosis factor (TNF) may have either a beneficial or a detrimental role in wound healing. Control and doxorubicin-treated (6 mg/kg, intravenously) rats underwent paired dorsal 5-cm linear wounds and had either vehicle or recombinant (r)TNF (0.5, 5, or 50 μg) applied locally to the wound. Paired wounds were harvested at 7 and 14 days after wounding and analyzed for wound-bursting strength (WBS) and activity of the gene for type 1 collagen and TNF. Doxorubicin treatment decreased WBS at 14 days but not at 7 days after wounding. Local application of 50 μg of rTNF decreased WBS in saline-treated rats and concentrations of 5 and 50 μg decreased WBS in doxorubicin-treated rats when measured 7 days after wounding. These effects dissipated when WBS was measured 14 days after wounding. Doxorubicin decreased wound collagen gene expression and local TNF treatment decreased wound collagen gene expression in saline-treated rats and further decreased it in doxorubicin-treated rats. The decrement in collagen gene expression induced by rTNF increased as the local dose of rTNF increased. The gene for TNF was not detectable in wounds from normal or doxorubin-treated rats at 3, 7, 10, or 14 days after wounding. These data suggest that the gene for TNF is not expressed in wounds and that the local application of TNF is detrimental to wound healing as it decreases WBS and activity of the gene for collagen.

From the Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

TUMOR NECROSIS FACTOR-α (TNF), a 17-kd polypeptide produced by macrophages in response to endotoxin, is a highly conserved molecule that appears to play a major role in the pathogenesis of gram-negative shock.[1] It is also named cachectin because it has many metabolic effects that may result in the wasting syndromes that accompany chronic disease states.[2] In addition studies have shown that TNF has potent, highly specific effects on many different types of cells.[3-8]

Tumor necrosis factor stimulates fibroblast proliferation,[3] prostaglandin production, and collagenase gene expression.[4] The ability of TNF to stimulate fibroblast proliferation may be due, in part, to its ability to promote production of interleukin-6 (IL-6) by fibroblasts[5] because IL-6 or B2-interferon is known to function as an autocrine mediator of cell proliferation in fibroblasts. Tumor necrosis factor stimulates the expression of endothelial cell surface antigens, including intercellular adhesion molecule-1 (ICAM-1), that modulate the adherence of inflammatory leukocytes to endothelial cells.[6] Tumor necrosis factor is chemotactic for macrophages,[7] and has been shown to activate macrophages *in vitro*.[8] It stimulates angiogenesis when implanted in rat cornea and when applied to developing chick chorioallantoic membrane.[9] It has been shown to induce the release of growth factors, including platelet-derived growth factor, known to be involved in the early phases of wound healing.[10]

In vitro the action of TNF has been elucidated at the molecular level. Tumor necrosis factor appears to increase protein kinase C activity in fibroblasts, resulting in prolonged activation of jun gene expression, increased activator protein-1 (AP-1) activity, and induction of AP-1 responsive genes, including collagenase, resulting in a marked increase in collagenase transcription.[11] The ability of TNF to stimulate fibroblasts to repopulate a wound space, attract macrophages and other inflammatory cells to a site of tissue injury, and to promote angiogenesis makes it a prime candidate for a role as a mediator of the cellular response to tissue injury.[12] However contrary to the *in vitro* evidence supporting a role for TNF in normal wound healing is the fact that TNF inhibits collagen gene expression in cultured fibroblasts.[13] Although increased collagenase gene expression and decreased collagen synthesis induced by TNF may have a role in tissue remodeling,[14] this combination is not likely to be beneficial as

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Address reprint requests to Jeffrey A. Norton, M.D., Head, Surgical Metabolism Section, Surgery Branch, National Cancer Institute/NIH, Building 10, Room 2B07, Bethesda, MD 20892.

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an early promoter of healing. Whether TNF acts as a mediator of the local response to tissue injury and how it affects collagen or collagenase synthesis *in vivo* is still unknown.

Recent work using a sponge matrix model suggested that TNF may be present in normal wound fluids but levels were minimal (1.4 U/mL) and may not have biologic relevance.[15] Furthermore contradictory evidence exists regarding whether local application of TNF to wounds improves healing. One recent study[16] indicates that it does, while another recent study[17] suggests that it does not. In light of *in vitro* evidence for a beneficial effect of TNF on wound healing[3-13] and contradictory *in vivo* evidence,[15-17] we performed the following study to determine if the gene for TNF was expressed in wounds and to identify the effects of local application of recombinant human TNF (rTNF) on normal and doxorubicin-impaired healing.

Materials and Methods

Materials

Recombinant human TNF was a gift of Cetus Corporation (Emeryville, CA) and had a specific activity of 10^7 U/mg, as measured in the L929 bioassay and an endotoxin level of 30 pg/2.5 × 10^6 U as measured by a standard limulus assay.[18] The human type I α (1)—collagen cDNA probe and the human glyceraldehyde-phosphate dehydrogenase (GAPDH) cDNA probe all have been described previously.[19] The human TNF-α probe also has been described before.[20] Phosphate-buffered saline (PBS) was purchased from Biofluids (Rockville, MD). Doxorubicin (Adriamycin, Adria Laboratories, Dublin, OH) was purchased from Farmitalia Carlo Erba (Milan, Italy).

General Wound Model

Adult Male Fischer 344 rats weighing between 150 to 200 g were anesthetized with 35 mg/kg sodium pentothal (intraperitoneally) and shaved. Five-centimeter paired linear dorsal incisions were made 2 cm from the midline. The wounds were dressed with sterile absorbable collagen sponges (Helistat, US Surgical Corp., Norwalk, CT) into which rTNF (0.5, 5, 50 μg in 0.2 mL PBS with 0.5% bovine serum albumin [BSA]) or vehicle alone (PBS with 0.5% BSA) was placed, and covered with Bioclusive transparent dressings (Johnson and Johnson Products Inc., New Brunswick, NJ) that were held in place with Mastisol, (Ferndale Laboratories, Ferndale, MI) and skin staples (Accustaple, Deknatel, Division of Howmedica, Queens Village, NY). Animals then each received an intravenous injection of doxorubicin (6 mg/kg) or an equivalent volume of 0.9% sodium chloride *via* the dorsal penile vein. Animals were killed at designated intervals by sodium pentothal overdose. The pelts were harvested and a 1 cm × 3 cm wound specimen was taken from the middle of the wound for wound-bursting strength (WBS), using a standard cutting form. Wound-bursting strength was measured on a Tensiometer (Instron, Canton, MA) at a velocity of 10 cm/min and was expressed in grams. The remaining part of the wound was excised and immediately placed in liquid nitrogen for RNA studies. Wound specimens were stored at −70 C.

In the initial experiment, 51 rats were wounded and randomly allocated to either the doxorubin or saline treatment groups and had one of three doses of rTNF (0.5, 5.0, and 50.0 μg) applied locally to one of the wound pair. These animals were each killed 7 days later. In a second experiment, 40 rats were wounded and allocated to either doxorubicin or saline treatment and two possible doses of rTNF (5.0 and 50 μg) applied locally as before. These animals had WBS measured 14 days later. In a final experiment, 40 rats were wounded and randomly treated with either doxorubicin and saline and one of three possible doses of rTNF applied locally to one of the wound pair. Wounds were harvested for RNA analysis 3, 7, 10, and 14 days after wounding.

Northern Blotting and Processing of Wounds for RNA

Total RNA extraction was a modification of the method of Chirgwin et al.[21] Frozen wound tissue (0.1 to 0.5 g) was ground into a fine powder using a mortar and pestle, homogenized in guanidine isothiocyanate buffer, placed on a cesium chloride gradient, and centrifuged overnight in a SW41 rotor (Beckman Instruments, Palo Alto, CA) at 30,000 RPM. The RNA pellet was taken up in 0.3 mol/L sodium acetate and precipitated with chilled, absolute ethyl alcohol. The resulting pellet was resuspended in autoclaved, deionized, distilled water. RNA concentration and purity was evaluated using a Beckman DU-50 spectrophotometer (Beckman Instruments) and a minigel. Thirty micrograms total RNA was loaded on a 1% Agarose gel containing 2.2 mol/L formaldehyde and run at 20 mV overnight. Gels were stained with ethidium bromide and then transferred to nitrocellulose filters (Bethesda Research Labs, Gaithersburg, MD) by capillary blotting in 10X sodium chloride sodium citrate (SSC) buffer. The nitrocellulose filters were then vacuum baked for 3 hours. The filters were prehybridized for 2 to 4 hours at 42 C in prehybridization solution containing formamide, 5X SSC, TRIS buffer, Denhart's solution, and Salmon Sperm DNA (from 5'3', West Chester, PA) 20 μg/mL. Probes were labeled with P^{32} by the Random Primer labeling method (Lofstrand Labs, Rockville, MD) and filters were then hybridized overnight using 2 × 10^7 CPM of labeled probe (specific activity 5 × 10^6 CPM/25 ng) in hybridization solution containing the above prehybridization solution components as well as 10% dextran sulfate. Filters were then washed extensively with 1X SSC with 0.1% sodium dodecyl sulfate and finally with 0.5% SSC. Filters were then exposed to x-ray film at −70 C with an intensifying screen. The films were processed and scanned using a Beckman DU-8 Spectrophotometer (Beckman Instruments). The collagen mRNA index was calculated as described previously[19] by normalization with GAPDH for loading differences and comparison to FS-4 fibroblast or normal rat skin as a positive control because these cells express mRNA for the type 1 collagen gene. RNA from peritoneal exudative cells stimulated with endotoxin and probed for TNF served as the positive control for the TNF gene.

Cell RNA Samples

FS-4 fibroblasts were a gift from Dr. Jan Vilchek, New York University, New York, NY. Two large flasks of cells grown to confluence were harvested and washed in sterile, autoclaved PBS. Cells were resuspended in guanidium isothiocyanate (GIT) buffer, vortexed for 2 minutes, and then processed for RNA extraction as above and probed as for the tissue specimens.

Rat thioglycollate elicited peritoneal macrophages were harvested as previously described[22] and incubated for 2 hours at 37 C in the presence of lipopolysaccharide (10 μg/mL). Cells were then processed for RNA as described above.

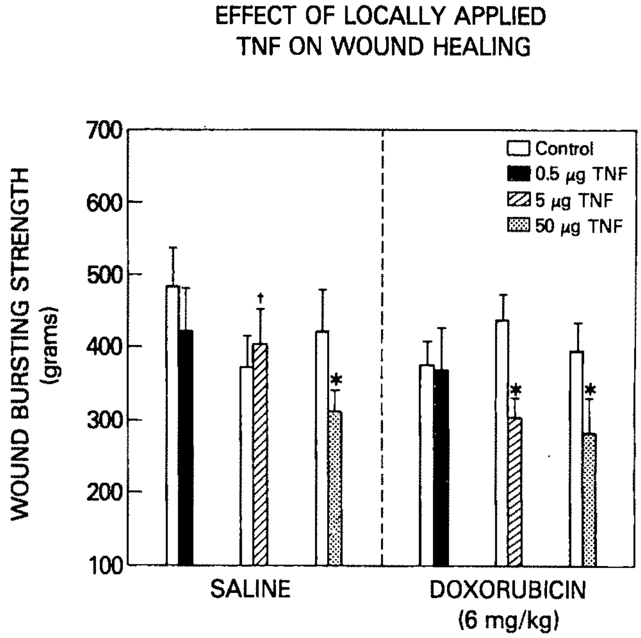
Statistics

Data are presented as mean ± standard error of the mean and compared by dependent and independent Student's t test.

Results

Wound-bursting Strength

Recombinant TNF decreases WBS at 7 days in saline-treated animals when rTNF was applied locally at a dose of 50 μg ($p < 0.05$) (Fig. 1). There was no effect on WBS in saline-treated animals at local rTNF doses of 0.5 or 5 μg. In the doxorubicin-treated animals, there was a significant decrease in WBS at local rTNF doses of 5 and 50 μg ($p < 0.05$, Fig. 1). Seven days after wounding there were no differences in WBS of control wounds from saline-treated rats compared to the same wounds of doxorubicin-treated rats (Fig. 1). However locally applied TNF at a dose of 5 μg significantly decreased WBS in doxorubicin-treated rats compared to saline-treated animals that received the same dose of local rTNF ($p < 0.05$) (Fig. 1). At 14 days after wounding there were no significant differences in WBS of wounds treated locally with either dose of rTNF compared to paired control wounds treated with buffered vehicle (Fig. 2). At 14 days rats previously given intravenous doxorubicin had significantly reduced WBS compared to animals given saline (Fig. 2). The same doxorubicin-induced reduction in WBS was present with or without locally applied rTNF (Fig. 2).



* Experimental<Control p<0.05
† Saline>D p<0.05

FIG. 1. Effect of locally applied TNF on wound-bursting strength (WBS) of 7-day wounds. Rats were treated with either saline (left panel) or doxorubicin (right panel) and wounded. Either vehicle or rTNF (0.5, 5, or 50 μg) was applied locally to paired wounds on the day of wounding. Wound bursting strength in grams was measured 7 days after wounding. *The local application of 50 μg of rTNF in saline-treated rats significantly reduced WBS compared to vehicle-treated paired wounds ($p < 0.05$). *The local application of either 5 or 50 μg of TNF in doxorubicin-treated rats significantly reduced WBS compared to vehicle-treated paired wounds ($p < 0.05$). †Doxorubicin administration significantly decreased WBS in wounds treated locally with 5 μg of TNF ($p < 0.05$).

Wound RNA

Northern blots of RNA from FS-4 fibroblasts (Fig. 3A) and normal rat skin (Fig. 3B) hybridized against a specific cDNA probe for type I collagen demonstrate the presence of message and serve as a positive control. The collagen message is not expressed by macrophages that serve as a negative control (Fig. 3B). Wounds from doxorubicin-treated rats (dox) have decreased levels of collagen gene expression compared to wounds from saline-treated animals (con) (dox 7 days *versus* con 7 days, Fig. 3A and dox 7 days *versus* con 7 days, Fig. 3B). When normalized for inadvertent loading variability by comparing it to expression of the gene for GAPDH (collagen index), the reduction in collagen gene expression by doxorubicin is approximately 40%. Local rTNF treatment decreased collagen gene expression in wounds from saline-treated animals at doses of 0.5, 5.0, and 50 μg (sal 7 days/TNF 50, sal 7 days/TNF 5, and sal 7 days/TNF 0.5 *versus* CON

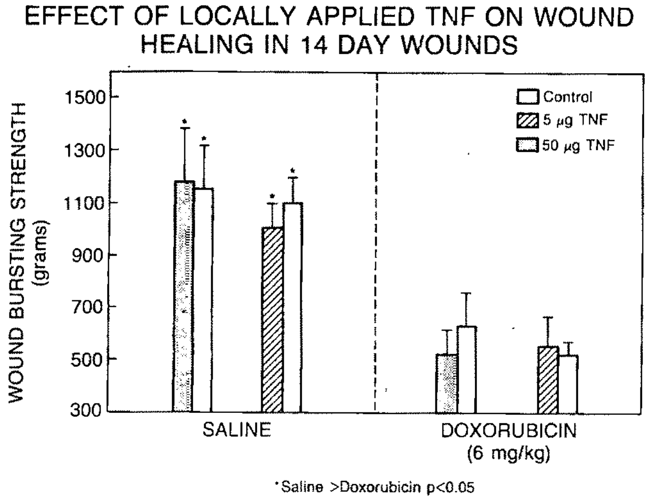


FIG. 2. Effect of locally applied TNF on WBS of 14-day wounds. Rats were treated as in Figure 1 except that only two doses of local rTNF were used, either 5 or 50 μg. Local application of either dose of rTNF at wounding did not affect WBS measured 14 days later. Systemic administration of doxorubicin (6 mg/kg IV) significantly reduced 14-day WBS whether the wound was treated locally with vehicle or rTNF ($p < 0.05$).

7 days, Fig. 3B). When adjusted for loading differences, the negative effect of rTNF on collagen gene expression appeared to be dose related: as the dose of rTNF increases the amount of collagen gene expression decreases (Fig. 3b). In doxorubicin-treated rats there is also a dose-related decrease in collagen gene expression with local rTNF treatment at doses of 5 (dox 7 days/TNF 5 *versus* dox 7 days, Fig. 3B) and 50 μg (dox 7 days/TNF 50 *versus* dox 7 days, Fig. 3A). The reduction in collagen gene expression with the combination of 50 μg of local TNF and intravenous doxorubicin was so profound that collagen gene expression is barely detectable 7 days after treatment at a time when control wounds have a collagen index greater than 2 (Fig. 3A).

Northern blot analysis for the expression of the TNF gene (Fig. 4) is positive only for the endotoxin-stimulated macrophages that are known to produce TNF[22] and serve as a positive control. RNA from wounds of saline-treated or doxorubicin-treated rats demonstrate no evidence for the expression of the gene for TNF (Fig. 4).

Discussion

Despite the previous reports of *in vitro* (fibroblast proliferation, chemotaxis, and angiogenesis)[3-10,12] and *in vivo*[16] data that suggest that TNF may improve wound healing, the present data suggest that exogenous rTNF applied locally to wounds impairs both WBS and collagen gene expression. The detrimental effects of TNF on WBS were apparent in both saline- and doxorubicin-treated animals 7 days after wounding. In doxorubicin-treated rats a decrease in WBS was seen with a lower dose of rTNF than in saline-treated rats (Fig. 1), which suggests that the deleterious effects of local TNF on WBS may be even more substantial during conditions that are known to impair healing. This observation further indicates that local application of rTNF will not be useful to correct healing in conditions associated with impaired healing. In fact the use of it in these conditions may have the opposite effect and further worsen the healing process.

Local application of TNF had no apparent effect on bursting strength 14 days after wounding in either saline- or doxorubicin-treated rats, suggesting that the initial reduction in WBS seen with locally applied TNF may be reversible with time (Fig. 2). The results of northern blot analysis for the gene for type-1 collagen also indicated

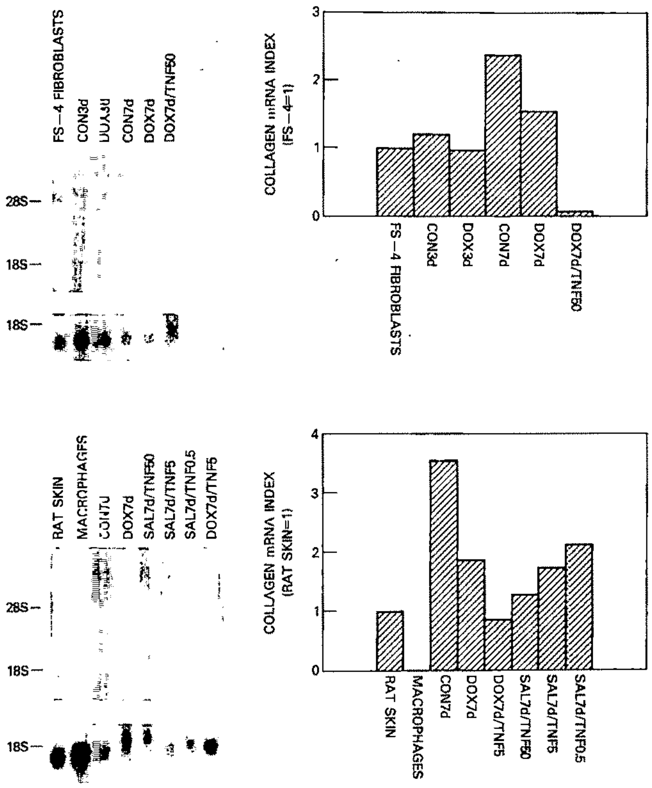


FIG. 3A and 3B. Collagen gene expression in wounds. A, (top) Gel (left) and Calculated collagen mRNA index (right) for wounds harvested at either 3 or 7 days after wounding normalized to FS-4 fibroblasts (positive control) by calculation of the collagen mRNA index. Gels probed with cDNA for type-1 collagen and glyceraldehyde-phosphate dehydrogenase GAPDH. Collagen mRNA expression is presented as an index by normalization with GAPDH for loading differences and comparison with FS-4 cells (index = 1). Abbreviations used: CON = control, 3d or 7d postwounding day 3 or 7, respectively, DOX = doxorubicin, TNF 50 = 50 μg tumor necrosis factor applied to the wound. B, (bottom). Same as A except rat skin is positive control and macrophages are negative control. Collagen mRNA expression is presented as an index by normalization with GAPDH for loading differences and comparison with normal rat skin (index = 1). Abbreviations used: same as A except SAL = saline-treated IV, TNF 0.5, 5, and 50 = 0.5, 5, and 50 μg of TNF applied to the wound.

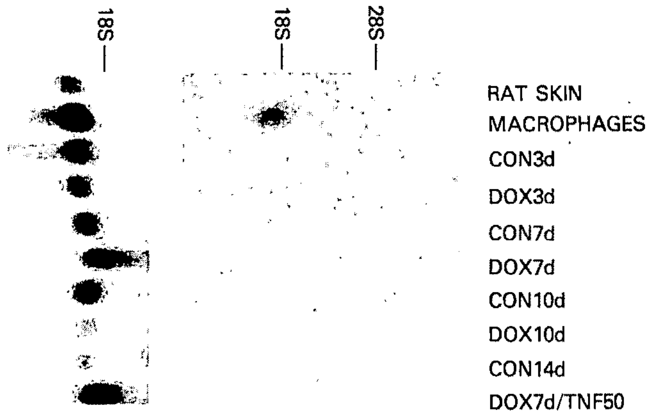


FIG. 4. Tumor necrosis factor gene expression in wounds. Gel demonstrating TNF-α gene expression in rat skin, macrophages treated with endotoxin and various treated wounds 3, 7, 10, and 14 days after wounding. Gels probed with cDNA for TNF and cDNA for GAPDH. Despite the fact that each lane had RNA present (blot) with GAPDH probe), only macrophages had evidence of the gene for TNF (positive control). The TNF gene was not expressed in normal rat skin or in any wound tested. Abbreviations used: CON = control, DOX = doxorubicin treated, 3, 7, 10, 14 d = 3, 7, 10, 14 days after wounding, and TNF 50 = wound treated locally with 50 μg TNF.

that the local application of rTNF to wounds reduced the expression of the gene for collagen in a dose-dependent manner (Fig. 3). This effect was again additive with doxorubicin in that doxorubicin also decreases the expression of this gene but the application of rTNF decreased it further. This finding suggests that one mechanism of rTNF's reduction in WBS is decreased synthesis of collagen, the basic protein element in healing. The application of TNF to fibroblast cultures *in vitro* also has resulted in decreased collagen gene expression.[13] In other words rTNF appears to have a direct inhibitory effect on the gene that controls new collagen synthesis.

Northern blots for collagen also demonstrated that 7 days after wounding doxorubicin-treated animals had decreased levels of collagen mRNA expression relative to saline-treated controls (Figs. 3A and B), despite the fact that the WBS were comparable (Fig. 1). This reduction in new collagen synthesis resulted in a decrease in WBS of doxorubicin-treated rats (compared to saline-treated controls) 14 days after wounding (Fig. 2).

When the present observations that local application of rTNF can reduce WBS and collagen gene expression are combined with the fact that the message for TNF is not present in either normal or doxorubicin-treated wounds (Fig. 4), it is difficult to suggest that the paracrine action of TNF may be used as a strategy to improve impaired healing. Rather these results suggest that TNF is not a local factor in normal or chemotherapy-impaired wound healing and that conditions resulting in high local levels of TNF may cause reduced collagen gene expression and impaired healing. Pathologic conditions that are known to result in elevated serum levels of TNF also are associated with impaired wound healing. These include gram-negative endotoxic shock,[23,24] acquired immune deficiency syndrome,[25] meningococcemia,[26] and cancer.[27] In addition, during recent investigations in our laboratory, we administered sublethal systemic doses of rTNF to rats and measured WBS. As expected from the current results, we found that systemically administered rTNF significantly reduced WBS when administered twice daily for 7 days after wounding (unpublished observations). This finding is consistent with the hypothesis that disease states associated with elevated circulating levels of TNF may mediate a healing impairment through the presence of systemic levels of TNF. However further investigations will be necessary to determine unequivocally if the wound healing impairment associated with these conditions is, in fact, mediated through an effect of TNF.

Despite previous reports of *in vitro*[3-10,12] and *in vivo*[16] evidence that suggests that tumor necrosis factor may be an important acute mediator of healing[12] and that the addition of exogenous TNF may improve impaired healing,[16] the present results suggest that the gene for TNF is not expressed in either normal or doxorubicin-impaired wounds and that when exogenous TNF is added to wounds it reduces the expression of the gene for type I collagen and it reduces the force required to disrupt the wound. These changes appear to be amplified by the concurrent administration of doxorubicin, a substance known to inhibit healing. Our results suggest that the local application of TNF will not be useful to improve impaired healing.

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Interleukin-2 Induces Early Multisystem Organ Edema Mediated by Neutrophils

RICHARD WELBOURN, M.B., F.R.C.S.,* GIDEON GOLDMAN, M.D.,* LESTER KOBZIK, M.D.,†
IAN PATERSON, M.B., F.R.C.S.,* DAVID SHEPRO, PH.D.,‡ and HERBERT B. HECHTMAN, M.D.*

From the Departments of Surgery and Pathology,† Brigham and Women's Hospital, Harvard Medical School, and the Biological Science Center,‡ Boston University, Boston, Massachusetts*

Interleukin-2 (IL-2), an agent known to activate neutrophils (PMN) with thromboxane (Tx)B_2 release, produces pulmonary edema within 6 hours of intravenous infusion. This study tests the role of PMN in mediating the edema. Anesthetized rats received 10^6U recombinant human IL-2 ($n = 15$) or vehicle ($n = 14$) as a constant intravenous infusion during a period of 1 hour. At this time there was leukopenia 3.63 ± 0.43 ($x10^3/mm^3$) relative to vehicle-infused control rats 6.12 ± 0.86 and a decline in PMN, 2.19 ± 0.14 relative to control value of 3.33 ± 0.05 (both $p < 0.05$). After 6 hours edema, as measured by increase in the wet to dry weight (W/d) ratio, was present in the lungs (4.93 ± 0.20 relative to control 4.06 ± 0.10), heart (4.09 ± 0.11 *versus* 3.76 ± 0.08), liver (3.50 ± 0.10 *versus* 3.18 ± 0.10), and kidney (4.25 ± 0.07 *versus* 4.00 ± 0.07) (all $p < 0.05$). There was increased lung permeability demonstrated by bronchoalveolar lavage fluid protein concentration of 1970 ± 210 μg/mL relative to control 460 ± 90 μg/mL ($p < 0.05$). Interleukin-2 resulted in lung PMN sequestration of 53 ± 7 PMN/10 high-power fields (HPF) relative to 23 ± 2 PMN/10 HPF in controls ($p < 0.05$) and increased plasma TxB_2 levels to 1290 ± 245 pg/mL relative to control 481 ± 93 pg/mL ($p < 0.05$). Pretreatment of other rats ($n = 8$) with selective anti-rat neutrophil antiserum 18 hours before the experiment led to a peripheral PMN count 10% of baseline and prevented edema in the lungs (W/d ratio 4.20 ± 0.16) and heart (3.67 ± 0.07) (both $p < 0.05$) but not liver or kidney. Protein in lung lavage was reduced to 760 ± 220 μg/mL ($p < 0.05$). The protection afforded by leukopenia was associated with lack of PMN sequestration and prevention of the increase in plasma Tx levels (484 ± 120 pg/mL, $p < 0.05$). These data indicate that the rapid induction of lung and heart edema with a 1-hour infusion of IL-2 in the rat is mediated, in large part, by activated PMNs.

NEUTROPHILS (PMN) ARE IMPORTANT mediators of early tissue injury in various inflammatory states, such as those triggered by ischemia, sepsis, acid aspiration, and trauma.[1-5] Neutrophil-dependent injury is characterized by the adhesion of activated PMNs to endothelium and their release of eicosanoids, free radicals, and proteases with resultant edema formation.[6,7] Neutropenia has been shown to attenuate injury in these settings.

Interleukin-2 (IL-2) causes a vascular leak syndrome, known to be mediated, in part, by lymphocyte activation after several days.[8-11] However patients given IL-2 develop edema within 24 hours of injection, suggesting involvement of nonlymphocyte-dependent mechanisms.[12] Thus IL-2 activates PMNs within 4 hours, causing the generation and release of thromboxane (Tx)A_2 and probably other vasotoxic agents such as free radicals and neutral proteases.[13] Because IL-2 produces increased permeability within 6 hours of intravenous infusion, we postulated the involvement of PMNs in this event.[13,14] This study was designed to investigate the inflammatory role of PMNs in mediating early permeability after intravenous IL-2 infusion in rats.

Materials and Methods

Animal Preparation

Thirty-seven adult male Sprague-Dawley rats (Charles River Labs., Wilmington, MA) weighing approximately 500 g were anesthetized with intraperitoneal ketamine (35 mg/kg). A jugular venous catheter was inserted for fluid or drug infusion (1 mL/hr) and hourly intravenous anesthetic dosing (ketamine 8 mg/kg; xylazine 1 mg/kg). All animals were maintained in the supine position for the duration of the experiment.

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Address reprint requests to Herbert B. Hechtman, M.D., Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

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Preparation of Test Solutions

Recombinant human IL-2 (Hoffman La Roche, Nutley, NJ) was obtained as lyophilised powder and reconstituted just before use with 1 mL normal saline per 10^6U IL-2. The saline vehicle contained mannitol (5 mg/mL) and human albumin (25 mg/mL). Endotoxin content was 3.3 pg/10^6U IL-2 measured by the Limulus amebocyte lysate (Associates of Cape Cod, Falmouth, MA).

Rabbit Anti-rat Neutrophil Antiserum

Selective neutropenia was accomplished according to methods previously described.[15] Briefly, rat neutrophils were recruited by intraperitoneal injection of 1% glycogen. Four hours later the peritoneum was lavaged with phosphate-buffered saline (PBS) and the return was centrifuged and resuspended. The cellularity of the lavaged cells was 99% neutrophils measured on a hemocytometer. Half-strength PBS was added for 30 seconds to lyse any contaminating erythrocytes and the neutrophils injected intradermally into rabbits. Following an identical challenge 3 weeks later, rabbit serum was collected after another week and frozen at −20 C until use. This rabbit serum was then injected intraperitoneally into rats (0.5 mL/100 g body weight) 18 hours before the experiment.

Prostanoid Assay

Concentrations of TxB_2 and 6-keto-$PGF_{1\alpha}$ in plasma were measured in duplicate with a double-radioimmunoassay kit using an antibody whose cross-reactivity with heterologous prostanoids was less than 1% (Seragen, Cambridge, MA).[16]

Experimental Protocol

Three groups were studied: 10^6U IL-2 ($n = 9$), vehicle ($n = 8$), or 10^6U IL-2 in animals pretreated with anti-neutrophil serum ($n = 8$). The IL-2 was infused by pump during a 1-hour period (model 352, Sage Instruments, Orion Research Inc., Cambridge, MA). Six hours after the beginning of the experiment, animals were killed with an overdose of anesthetic. A thoracotomy was performed and blood for eicosanoid assay was obtained by cardiac puncture and was collected into tubes containing 0.3 mL 0.07 mol/L ethylenediamine tetracetic acid and 0.09 mol/L aspirin, centrifuged at 1500*g* for 20 minutes (PR-2, International Equipment Co., Needham Heights, MA) and the plasma stored at −20 C until assayed for prostanoids. The left lung bronchus was clamped. Bronchoalveolar lavage (BAL) of the right lung was performed with 3 mL saline. This was repeated three times. The combined lavage return of about 8 mL was centrifuged at 1500 *g* for 20 minutes at 4 C, frozen at −20 C, and subsequently used for assay of protein concentration using the spectrophotometric dye method.[17]

The wet-to-dry (W/d) ratios of the left upper lung lobe, heart, left lobe of the liver, left kidney, and spleen were calculated after weighing the freshly harvested organ, heating at 90 C in a gravity convection oven (Precision Scientific Group, Chicago, IL) for 72 hours, and weighing the residuum.

The left lower lung lobe was used for histology. After the animals were killed, the lobe was perfused with 10% formaldehyde and then inflated with the same material to a pressure of 25 cm H_2O. After fixation the inferior aspect of the lobe was stained with hematoxylin and eosin for light microscopic analysis. All microscopic sections were interpreted in a blind fashion by a pulmonary pathologist (L Kobzik). Lung leukosequestration was quantitated by counting alveolar septal wall PMNs. Only peripheral lung parenchyma was examined. Microscopic fields containing other structures such as airways, large vessels, and pleura were excluded. Leukocyte entrapment was expressed as the mean number of PMNs per 10 high-power fields (HPF; ×1000).

To assay circulating white blood cells, additional animals were infused with 10^6U IL-2 ($n = 6$) or vehicle ($n = 6$) for 1 hour. At this time animals were killed, thoracotomies were performed for cardiac puncture, and blood was sampled for estimate of white cell count (performed on a hemocytometer) and differential count.

Results are expressed as mean ± standard error in text and figures. Statistics were conducted by an analysis of variance and if this was positive, a nonpaired Student's t test. Significance was accepted if $p < 0.05$.

Animals in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and the Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (Department of Health, Education and Welfare, Publication No. 78-23 (National Institute of Health, revised, 1978).

Results

At the end of the 1-hour infusion of 10^6U IL-2 there was leukopenia $3.63 \pm 0.43 \times 10^3/mm^3$ relative to vehicle-infused rats $6.12 \pm 0.86 \times 10^3/mm^3$, with a decrease in PMNs to $2.19 \pm 0.14 \times 10^3/mm^3$, fewer than $3.33 \pm 0.05 \times 10^3/mm^3$ in controls (both $p < 0.05$). After 6 hours edema was present in the lungs (W/d ratio 4.93 ± 0.20 relative to 4.06 0.10 in controls); heart (W/d 4.09 ± 0.11 *versus* 3.76 ± 0.08); liver (W/d 3.50 ± 0.10 *versus* 3.18 ± 0.10); kidney (W/d 4.25 ± 0.07 *versus* 4.00 ± 0.07) (all $p < 0.05$, Fig. 1). There was increased permeability dem-

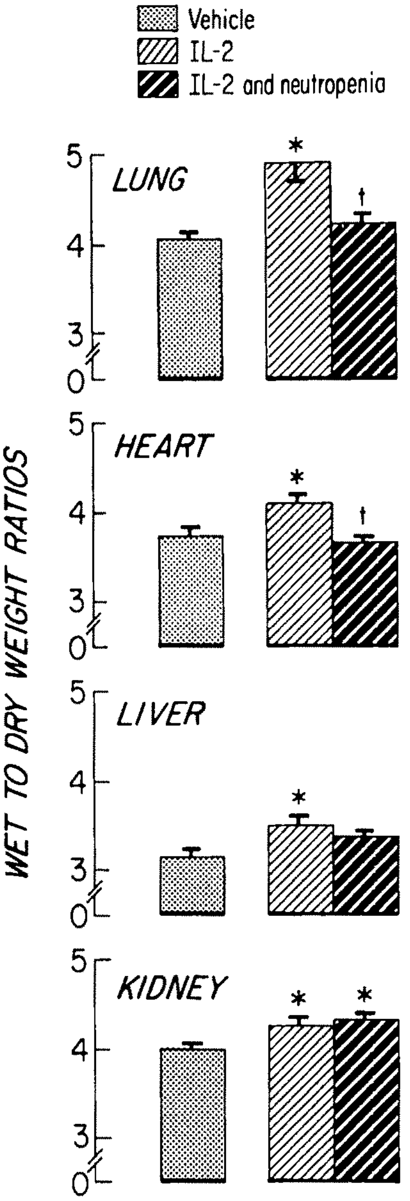

FIG. 1. A 1-hour infusion of 10^6 U produced multisystem organ edema after 6 hours. Selective neutropenia prevented the edema in lung and heart. Liver and kidney were not protected. * and † indicate $p < 0.05$ relative to vehicle and IL-2, respectively.

onstrated by BAL fluid protein accumulation of 1970 ± 210 μg/mL relative to 460 ± 90 μg/mL in vehicle-infused controls ($p < 0.05$, Fig. 2). Interleukin-2 infusion produced lung leukosequestration of 53 ± 7 PMN/10 HPF relative to 22 ± 3 PMN/10 HPF in control rats ($p < 0.05$, Fig. 3) and increased plasma TxB_2 to levels higher than in control animals ($p < 0.05$, Fig. 4). Levels of 6-keto-$PGF_{1\alpha}$ were unaffected by IL-2.

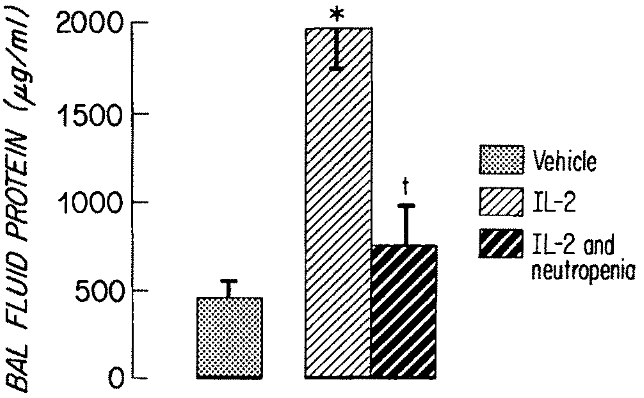

FIG. 2. The protein leak that resulted from IL-2 infusion during a period of 1 hour was reduced by selective neutropenia. * and † indicate $p < 0.05$ relative to vehicle and IL-2, respectively.

Pretreatment of rats with anti-neutrophil serum led to a baseline peripheral PMN count of 0.12 ± 0.06 PMN × 10^3/mm^3 18 hours after injection. The total white cell count was reduced from 6.96 ± 1.08 × 10^3/mm^3 to 3.22 ± 0.43 × 10^3/mm^3 ($p < 0.05$). Platelet numbers were unchanged. Six hours after the start of IL-2 infusion, the peripheral PMN count was 0.48 ± 0.10 PMN × 10^3/mm^3 relative to 4.70 ± 0.19 PMN × 10^3/mm^3 in normal rats ($p < 0.05$). Lung leukosequestration and plasma TxB_2 were reduced to control levels of 25 ± 3 PMN/10 HPF ($p < 0.05$, Fig. 3) and 484 ± 120 pg/mL ($p < 0.05$, Fig. 4), respectively. Neutropenia protected against IL-2-induced edema in the lungs (W/d 4.20 ± 0.16) and heart (3.67 ± 0.07) (both $p < 0.05$, Fig. 1). Liver edema was slightly and not significantly decreased (W/d 3.33 ± 0.05). Kidney edema was not reduced (4.34 ± 0.08). Protein leak in BAL fluid was lowered, 760 ± 220 μg/mL ($p < 0.05$).

Discussion

The data of this study indicate that neutrophils are important mediators of the multisystemic organ edema that

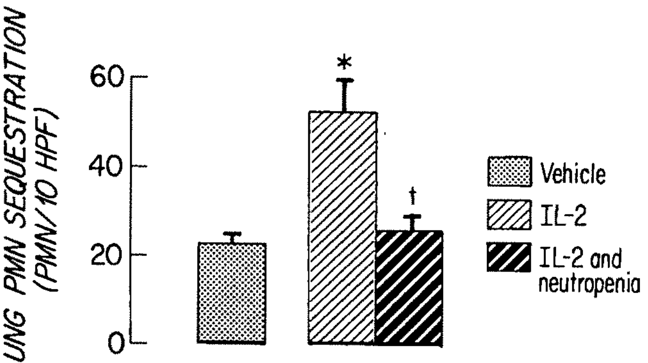

FIG. 3. Interleukin-2 produced lung neutrophil sequestration after 6 hours, which was reduced by selective neutropenia. * and † indicate $p < 0.05$ relative to vehicle and IL-2, respectively.

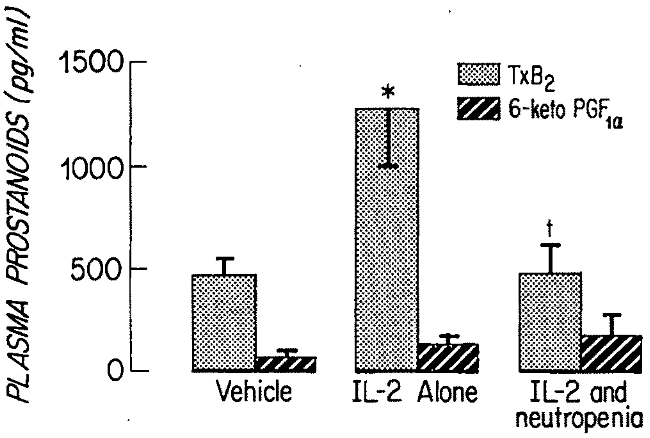

FIG. 4. Selective neutropenia prevented the increase in TxB_2 after IL-2 infusion. Interleukin-2 did not affect plasma 6-keto-$PGF_{1\alpha}$. * and † indicate $p < 0.05$ relative to vehicle and IL-2, respectively.

follows a 1-hour IL-2 infusion in the rat. This conclusion is based on the following observations: (1) PMNs were activated by IL-2 to synthesize TxB_2, as shown by the fact that neutropenia prevented TxB_2 synthesis; (2) there was relative neutropenia at the end of the IL-2 infusion consistent with the documentation of leukosequestration after 6 hours; (3) selective neutropenia prevented alveolar protein leak and lung and heart edema.

Interleukin-2 is known to produce lymphopenia, a marker of lymphocyte activation in patients.[18] This is followed by lymphocytosis after several days of therapy. Mild neutropenia to $2 \times 10^3/mm^3$ has been reported after IL-2 treatment in clinical trials.[18] Neutropenia is usually interpreted as an indication of neutrophil activation, reflecting cellular margination and adhesion in the peripheral vasculature.[19] In other settings of PMN-dependent endothelial injury their activation is accompanied by transient neutropenia, probably due either to upregulation or conformational change in adhesion glycoproteins (CD18 complex) or to microvascular plugging of activated cells, which have become stiff or clumped together.[5,20]

In other experimental studies, where there was a stimulus to PMN synthesis of TxB_2, there was also an increase in oxidative activity and neutropenia. For example in the first hour following reperfusion of the ischemic lower torso, neutropenia was observed and associated with an increase in PMN TxB_2 synthesis and H_2O_2 production PMN.[21-23] Interleukin-2 is known to stimulate PMNs to synthesize TxB_2.[13] The evidence that neutrophil depletion prevents the increase in plasma TxB_2 in this study suggests that the cellular source of Tx is PMN. Furthermore concentrations of IL-2 similar to those used in this study do not activate other circulating cells, such as lymphocytes or platelets, to synthesize TxB_2.[13] These considerations strongly suggest that in the present study neutrophils were activated either directly or indirectly by IL-2 to produce TxB_2 and possibly H_2O_2.

The ability of PMN to make the microvascular barrier more permeable has been noted in other studies, *i.e.*, reperfusion of the ischemic lower torso, intravascular complement activation, and acid aspiration, which all lead to PMN sequestration, leakage of protein into lung lymph and alveolar spaces, and lung edema.[2,5,24,25] In the current study, PMN sequestration in the lungs, accumulation of protein in BAL fluid, and lung edema have been documented. To our knowledge there has been no previous documentation of sequestration of PMNs in the lungs after IL-2 administration. Studies in which IL-2 has been given by repeated bolus or continuous injection have reported lung histologic findings only at 2 or 3 days, a time period unlikely to demonstrate involvement of PMNs in an acute inflammatory process.[26-28] However it is clear from other experimental models that factors in addition to PMNs also may contribute to organ injury following IL-2 infusion.[13,26,29]

We have little evidence to support a role for PMNs in mediating the liver and kidney edema after IL-2 infusion. Thus neutropenia did not improve the W/d ratio of the kidney. Edema in the liver was reduced, but not significantly. Both organs have been shown in previous studies to be injured after IL-2 administration.[8,11,26,30] In the setting of ischemic tubular necrosis, renal injury was reduced by the infusion of vasodilating prostaglandins.[31] Furthermore inhibition of Tx, thought to mediate, in large part, the functional and histologic damage following renal ischemia, only protected the ischemic kidney when renal vasodilatation was achieved at the same time. While *in vitro* studies suggest that the vasodilator PGI_2 is produced by endothelial cells with IL-2 stimulation, no observed increase in 6-keto-$PGF_{1\alpha}$ occurred in our study.[32] Thus a possible explanation for the lack of kidney protection is related to the low levels of vasodilating prostaglandins. An alternative explanation is that PMNs do not mediate IL-2-induced acute renal injury.

Neutrophils may cause endothelial injury *via* elastase and lysosomal enzyme release, generation of toxic oxygen radicals, or indirectly *via* release of vasoactive mediators such as Tx.[6,7] For PMNs to mediate injury directly, adhesion to endothelium appears to be a prerequisite. Thus, by pretreating with antibodies against CD18, PMN-dependent injury is prevented in other settings, such as hypovolemic shock and myocardial ischemia.[1,33] That CD18-dependent PMN adhesion might be involved in IL-2-induced injury is supported by the evidence that the intercellular adhesion molecule-1 (ICAM-1), the requisite ligand for CD11a/CD18, is induced *de novo* on skin biopsies in patients given IL-2.[34] However IL-2 is not capable of inducing the activation antigen ICAM-1 or another determinant of neutrophil adhesion, endothelial leukocyte adhesion molecule (ELAM-1) by itself; rather induction of further cytokines, IL-1, tumor necrosis factor (TNF), or gamma-interferon are necessary. Interleukin-2 has been shown to stimulate TNF production both *in vitro* and *in vivo*.[35-37] Tumor necrosis factor can induce *de novo* ICAM-1 and ELAM-1 within 6 hours.[38,39] Furthermore TNF is capable of activating neutrophils and directly increasing their adhesion to endothelium.[40,41] Recent evidence suggests that TNF actively participates in organ injury following IL-2 infusion.[42]

Interleukin-1 also can induce ICAM-1 and ELAM-1, and IL-1 synthesis is stimulated by TNF as well as IL-2.[43,44] Intercellular adhesion molecule-1 and ELAM-1 appear equally important in PMN–endothelial adhesion in this time period.[45] Interleukin-2 also produces gamma-interferon,[46] but this is probably not a mediator in our setting because lung PMN sequestration occurred within 6 hours of stimulus. This is less than the minimum period of 8 to 10 hours in which gamma-interferon can induce *de novo* ICAM-1 synthesis. Furthermore gamma-interferon does not induce ELAM-1.

In this study anti-neutrophil serum prevented the increase in plasma TxB_2. The reduction in organ injury may relate to an action of Tx in mediating the IL-2 injury. That Tx may be directly involved is supported by evidence from our and other laboratories that have demonstrated increases in pulmonary artery pressure within 6 hours of IL-2 administration.[14,47] Prevention of synthesis of the vasoconstrictor TxA_2 abrogated the pulmonary artery pressure increase and prevented the edema in isolated-perfused guinea pig lungs.[29] In the sheep lung lymph fistula preparation, the early (1 hour) but not later changes in lymph flow and lymph protein content were prevented by Tx inhibition.[13] Thromboxane also may modulate the microvascular barrier directly. In support of this, Tx mimic *in vitro* is capable of widening interendothelial junctions to increase the flux of fluid and macromolecules. Data are not available to clarify the indirect role of Tx as a cofactor together with PMNs in IL-2–induced early edema.

The data of this study suggest that the early edema produced by IL-2 infusion is mediated, in large part, by activated PMNs, the mechanism probably being adhesion to microvascular endothelium with release of vasotoxins.

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Acceleration of Superoxide Production from Leukocytes in Trauma Patients

HIROSHI TANAKA, M.D., HIROSHI OGURA, M.D., JUNICHIRO YOKOTA, M.D., HISASHI SUGIMOTO, M.D.,
TOSHIHARU YOSHIOKA, M.D., and TSUYOSHI SUGIMOTO, M.D.

From the Department of Traumatology, Osaka University Medical School, Osaka, Japan

Superoxide (O_2^-) and granulocyte elastase (GE) from neutrophils mediate host defense and tissue injury in inflammation. To determine alterations in leukocyte function after trauma, O_2^- production and GE secretion from neutrophils were studied in trauma patients (n = 20) and healthy controls (n = 15). The priming effect of tumor necrosis factor (TNF), interleukin-1α (IL-1α), and lipopolysaccharide (LPS) on O_2^- or GE release also was evaluated. Superoxide production (nmole/10 minutes) was elevated significantly in trauma patients at days 0 (9.5 ± 4.8), 1 (14.2 ± 7.3), and 3 (12.2 ± 5.9) and returned to control levels (4.2 ± 1.6) by day 7. There was no difference in GE secretion between trauma patients and healthy controls. Incubation of neutrophils with TNF induced release of both O_2^- and GE. Superoxide production was induced by TNF at concentrations at or greater than 10^{-11} mol/L. Granulocyte elastase secretion was induced in a time- and dose-dependent manner by TNF at concentrations between 10^{-10} and 10^{-7} mol/L. In contrast IL-1α and LPS did not potentiate O_2^- or GE release. These results suggest that neutrophil O_2^- production increases acutely in trauma. Tumor necrosis factor may mediate this O_2^- and GE production by neutrophils involved in the inflammatory response.

SEVERAL DEFECTS IN the host defense response occur after severe trauma. These include antibody response,[1] neutrophil chemotaxis,[2] delayed-type hypersensitivity, and neutrophil adherence.[3] Neutrophils play a central role in the host defense mechanism. They produce superoxide (O_2^-) and its derivatives and secrete neutral proteases such as granulocyte elastase (GE) and collagenase, which help mediate the host defense responses to inflammation. In particular these factors play a significant role in the degradation of matter recognized as foreign.[4] Superoxide, however, is a highly reactive compound that has been recognized as an important final mediator of tissue injury.[5] Granulocyte elastase also is believed to act on a wide variety of normal tissue substrates, such as clotting factors, fibronectin, and tissue components. Previously we reported that elevation of GE activity in inflammatory tissue may cause tissue injury in patients with septic shock complicated by multiple-organ failure.[6]

However the mechanism by which neutrophils are induced to secrete O_2^- and GE after trauma is not clearly understood. In addition clinical data on neutrophil capacity of O_2^- production or GE secretion after trauma has not been serially investigated. Recently several investigators reported that cytokines such as tumor necrosis factor (TNF) and interleukin-1α (IL-1α) may affect the activity of neutrophils.[7-9] Tumor necrosis factor, also known as cachectin, plays an important role in acute inflammation. Recombinant human TNF is known to stimulate, weakly but directly, the neutrophil respiratory burst and degranulation.[7] Interleukin-1α is also a powerful endogenous mediator of the immune response now thought to be responsible for many of the systemic changes that accompany the inflammatory response.[8] Interleukin-1α facilitates neutrophil chemotaxis *in vitro*. *In vivo* it may play a role, for example, in the recruitment of neutrophils seen after intradermal injection of endotoxin into rabbit skin.[9]

To clarify the alterations in leukocyte functions after trauma, we measured serially O_2^- production and GE secretion from neutrophils in trauma patients and healthy controls. We also studied whether TNF, IL-1α, and lipopolysaccharide (LPS) have a priming effect on O_2^- production and GE secretion.

Address reprint requests to Hiroshi Tanaka, M.D., Department of Anesthesiology, St. Louis University Medical Center, 3635 Vista Ave., P.O. Box 15250, St. Louis, MO 63110-0250.

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TABLE 1. *Patient Characteristics*

Number of patients	20
Average age (yrs)	37.0 ± 13.3
Age range	(16–65)
Gender	
Men	18
Women	2
Location of injury	
Head/neck	7
Face	3
Thorax	5
Abdomen	7
Extremities	13
Injury mechanism	
Motor vehicle accident	11
Fall	4
Other blunt	1
Penetrating	4
Injury Severity Score (ISS)	23.2 ± 10.6
ISS range	(9–50)

Patients and Methods

Patients

Neutrophils were isolated from blood drawn from 15 healthy controls and 20 consecutive trauma patients admitted to the Department of Traumatology, Osaka University Medical School, Osaka, Japan (Table 1). Superoxide production and GE secretion assays were performed within 24 hours of trauma (day 0), and on days 1, 3, and 7 thereafter.

Reagents

Recombinant human TNF (activity of 9.2 × 10^6 U/mg) was obtained from the Suntory Institute for Biomedical Research, Osaka, Japan. The material was homogenous (molecular weight = 18 kd) when analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE); and contained 0.49 ng/ml of endotoxin as determined by the limulus amoebocyte lysate (LAL) assay for undiluted preparations. Determination of TNF activity was based on an assay for cytotoxicity in a TNF-susceptible line of L-292 cells at 40 C, as described previously.[10]

Recombinant human IL-1α (2 × 10^7 U/mg) was obtained from the Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan. The material appeared homogenous (molecular weight = 18.3 kd) when analyzed by SDS-PAGE and contained 1 ng/mL of endotoxin as determined by the LAL assay. Interleukin-1α activity was determined by the thymocyte costimulation assay as described previously.[11]

Lipopolysaccharide from the *Escherichia coli* strain 0128:B12 was obtained from Difco Laboratories (Detroit, MI).

L-pyroglutamyl-L-prolyl-L-valine-p-nitroanilide (S-2484) was obtained from Kabi Diagnostica, Stockholm, Sweden. It was dissolved in dimethylsulphoxide and diluted with four volumes of distilled water to a final concentration of 2 mmol/L. Other chemicals used in this study, including cytochrome C, concanavalin A (Con A), cytochalasin D (Cyt D), superoxide dismutase (SOD), and α1-protease inhibitor (α1-PI) were purchased from Sigma Chemical Company (St. Louis, MO). Hank's balanced salt solution (HBSS) was purchased from Gibco (Grand Island, NY).

Neutrophil Preparation

Whole blood from healthy controls and trauma patients was drawn into a heparinized syringe. Then neutrophils were isolated by dextran sedimentation and hypotonic lysis, as reported previously.[12] The final cell suspension (2 × 10^6 cells/mL) was comprised of more than 85% neutrophils by Wright-Giemsa staining. Cell viability was more than 95% as determined by trypan blue exclusion.

Superoxide Production Assay

Superoxide production by neutrophils was measured by the modified cytochrome C reduction assay reported previously.[13] Briefly, neutrophils were suspended in 800 μL of HBSS, to which a 100-μL aliquot of various concentrations of TNF, IL-1α, LPS, or buffer alone (control) was added. After incubating for 30 minutes, 100 μmol/L of cytochrome C was added. Then absorbance was measured at 550 nm after adding 5 μL of Con A (10 mg/mL) and 5 μL of Cyt D (1 mg/mL). The absorbance at 550 nm was converted to nanomoles of cytochrome C reduced per minute by using the extinction coefficient 1.91 × $10^4 \cdot M^{-1} \cdot cm^{-1}$.

Granulocyte Elastase Secretion Assay

Neutrophils were suspended in 800 μL of HBSS, to which TNF, IL-1α, LPS, and buffer alone (control) were added at varying concentrations. After incubating for 10, 30, 60, and 120 minutes, neutrophil suspensions were centrifuged at 500*g* and the supernatant of each tube subdivided into the fractions required for each assay. Granulocyte elastase activity was assayed by a new method developed in our laboratory.[14] Briefly, 200 μL of buffer (0.1 mol/L TRIS, 0.96 mol/L NaCl, pH 8.3) was added to 200 μL of sample and the reaction initiated by adding 200 μL of the substrate solution containing S-2484 at 37 C. After incubation for 3 hours, diazotization was performed to enhance sensitivity by the addition of 500 μL of each of the following: 5.8 mmol/L sodium nitrite in 0.48 mol/L HCl, 26.3 mmol/L ammonium sulfamate, and 2.7 mmol/L N-1-naphthyl-ethylenediamine dihydrochloride. After diazotization absorbance was measured at 545 nm. Cytoplasmic lactate dehydrogenase (LDH) ac-

TABLE 2. *O_2^- Production and GE Secretion by Healthy Control Neutrophils*

	O_2^- production		GE Secretion (u/L)
	(nmol/10 min)	(nmol/20 min)	
Buffer	3.6 ± 1.9	10.4 ± 4.5	5.4 ± 3.7
TNF	11.9 ± 5.5*	22.5 ± 6.2*	22.8 ± 7.1*
IL-1α	4.4 ± 3.0	12.9 ± 7.4	6.7 ± 4.0
LPS	4.6 ± 2.7	10.5 ± 5.9	5.7 ± 2.8

O_2^- production and GE secretion by neutrophils from 15 healthy controls incubated in: buffer; TNF, 10^{-8} mol/L; IL-1α, 10^{-8} mol/L; and LPS, 10 μg/mL. Values are expressed as the mean ± SD.

* $p < 0.01$ as compared to incubation in buffer alone.

tivity was used to monitor neutrophil viability as described previously.[15]

Statistical Analysis

All values were expressed as the mean ± standard deviation. Statistical significance was determined by the Tukey-Kramer test. Differences were considered significant at the $p < 0.05$ level.

Results

O_2^- Production and GE Secretion in Healthy Controls

Table 2 shows the changes in O_2^- production and GE secretion in healthy controls after incubation with 10^{-8} mol/L TNF, 10^{-8} mol/L IL-1α, 10 μg/mL of LPS, or buffer alone. Tumor necrosis factor stimulated both O_2^- production and GE secretion. However IL-1α and LPS did not stimulate O_2^- production or GE secretion when compared to buffer alone. Lactate dehydrogenase activity did not change through the incubation period (less than 10 u/mL), indicating that the TNF-induced O_2^- production and GE secretion are functional and not cytotoxic to the neutrophils. In addition O_2^- activity was abrogated by SOD and GE activity was inhibited by α1-PI (data not shown). Figure 1 shows the dose–response relationship for TNF-induced O_2^- production. Superoxide production was increased similarly at TNF concentrations greater than 10^{-11} mol/L. Figure 2 shows the dose–response relationship for TNF-induced GE secretion. Granulocyte elastase secretion was dose dependent and was observed at TNF concentrations ranging from 10^{-10} mol/L to 10^{-7} mol/L. In addition GE secretion increased in a time-dependent manner during a period of 2 hours (data not shown). Interleukin-1α did not induce GE secretion at concentrations of 10^{-11} mol/L to 10^{-7} mol/L, nor did LPS at 10 ng/mL to 100 μg/mL (data not shown).

O_2^- Production and GE Secretion in Trauma Patients

Table 3 shows serial changes in O_2^- production by the neutrophils of trauma patients after incubation with 10^{-8} mol/L TNF, 10^{-8} mol/L IL-1α, 10 μg/mL of LPS, or buffer alone. Superoxide production was increased in trauma patient neutrophils stimulated with buffer alone on days 0, 1, and 3 compared to healthy control neutrophils, then returned to control levels by day 7 (Fig. 3). Similarly TNF induced O_2^- production by trauma patient neutrophils on days 1 and 3 compared to healthy controls. In contrast IL-1α and LPS did not stimulate neutrophil O_2^- production compared to neutrophils incubated with buffer alone (Table 3). Table 4 shows the serial changes in GE secretion by neutrophils of trauma patients. Granulocyte elastase secretion was induced strongly by TNF compared to buffer alone. However neutrophil GE secretion did not vary in trauma patients throughout their clinical course and did not differ from neutrophil GE secretion in healthy controls.

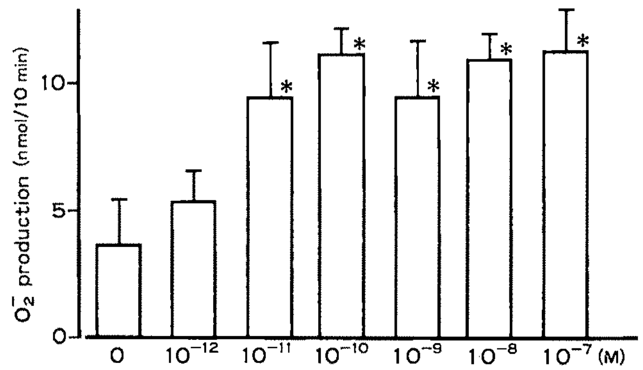

FIG. 1. Effect of TNF concentration on O_2^- production. Neutrophils (2 × 10^6 cells/mL) were incubated with varying concentrations of TNF for 30 minutes at 37 C. Data represent the mean ± SD in 15 healthy controls performed in duplicate. *$p < 0.01$ as compared to incubation in buffer alone.

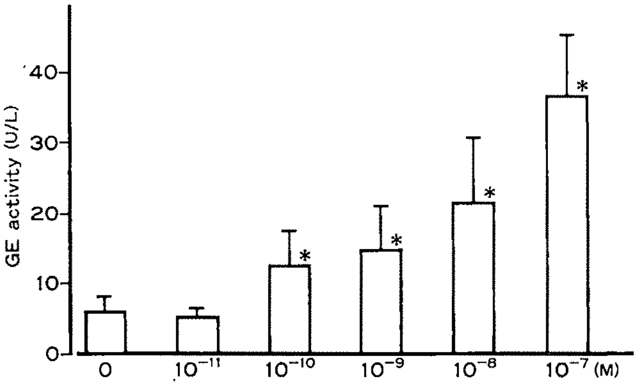

FIG. 2. Effect of TNF concentration on GE secretion. Neutrophils (2 × 10^6 cells/mL) were incubated with varying concentrations of TNF for 2 hours at 37 C. Data represent the mean ± SD in 15 healthy controls performed in duplicate. *$p < 0.01$ as compared with incubation in buffer alone.

TABLE 3. *Serial Changes in O_2^- Production by Trauma Patient Neutrophils*

	O_2^- Production (nmol/10 min)				
	Control	Day 0	Day 1	Day 3	Day 7
Buffer	3.6 ± 1.9	9.5 ± 4.8*	14.2 ± 7.3*	12.2 ± 5.9*	4.2 ± 1.6
TNF	11.9 ± 5.5	12.8 ± 5.3	17.5 ± 6.8†	17.2 ± 6.0†	9.4 ± 3.6
IL-1α	4.4 ± 3.0	11.3 ± 5.4*	15.7 ± 7.5*	13.3 ± 6.3*	4.6 ± 3.5
LPS	4.6 ± 2.7	9.8 ± 5.4*	16.3 ± 6.2*	14.1 ± 6.8*	4.7 ± 3.7

Serial changes in O_2^- production by neutrophils in 20 trauma patients incubated in: buffer, TNF, 10^{-8} mol/L; IL-1, 10^{-8} mol/L; and LPS, 10 μg/mL. Values are expressed as the mean ±SD.

* $p < 0.01$, † $p < 0.05$ as compared to healthy controls.

Discussion

Alterations of leukocyte function after trauma have been reported frequently. These phenomenon include serum-mediated depression of neutrophil chemiluminescence, decreased neutrophil chemotaxis,[2] and neutrophil heterogeneity.[16] In this study we demonstrate that increased O_2^- production is a characteristic alteration of leukocyte function in the early phase of trauma. Superoxide production in response to Con A and Cyt D was significantly increased in the neutrophils obtained from all trauma patients on days 0, 1, and 3. This increase normalized by day 7. In contrast there was no difference in GE secretion between trauma patients at any stage and controls. Solomkin[17] demonstrated that O_2^- production in response to formyl-methionyl-leucine-phenylalanine (FMLP) increased in trauma patients whose C3a levels were increased and who had an increased number of FMLP receptors. However O_2^- production was within normal levels in trauma patients with normal C3a levels and FMLP receptor numbers. Zimmerman et al.[18] reported that O_2^- concentrations in the pulmonary artery blood of four of eight adult respiratory distress syndrome (ARDS) patients were increased and suggested that abnormal O_2^- production caused pulmonary vascular injury and alveolitis. In our results O_2^- production was increased in all trauma patients during the early phase of trauma (the first 3 days). In addition the elevation of O_2^- production in the early phase of trauma is not dependent on the patient injury severity.

The mechanism responsible for increasing O_2^- production in the early phase of trauma is not known. The various mechanisms proposed involve or implicate neutrophil heterogeneity,[16] activation of receptors expressed on the neutrophil surface,[17] metabolic stimulation by stress hormones,[19] and other post-traumatic endocrine stimulation.[20] Solomkin et al.[17] demonstrated that binding of N-formylated peptides to their receptors on human neutrophils can induce a variety of cellular responses, including aggregation, chemotaxis, degranulation, and O_2^- production. Moon[20] measured NADPH oxidase and O_2^- production by polymorphonuclear leukocytes (PMN) in an *in vitro* endocrine milieu designed to simulate moderate trauma. They noted that exogenous glucose alone could enhance O_2^- production. Deitch et al.[19] demonstrated that a stress hormone cocktail containing epinephrine, cortisol, and glucagon provoked O_2^- production by neutrophils in response to zymosan. In this study we demonstrated that TNF in the range from 10^{-11} to 10^{-7} mol/L potentiated O_2^- production by neutrophils from healthy controls (priming effect) in response to Con A and Cyt D. Tumor necrosis factor at 10^{-10} to 10^{-7} mol/L also stimulated GE secretion. In addition no significant difference was observed between treated and untreated cells in the release of the cytoplasmic enzyme LDH, suggesting that TNF-induced O_2^- production and GE secretion is a functional, noncytotoxic process. We found that IL-1α (10^{-11} – 10^{-7} mol/L), and LPS (10 ng to 100 μg/mL) did not effect O_2^- production and GE secretion. Although clinical data on TNF are still scarce in the early phase of trauma, elevation of plasma TNF levels is known in inflammatory

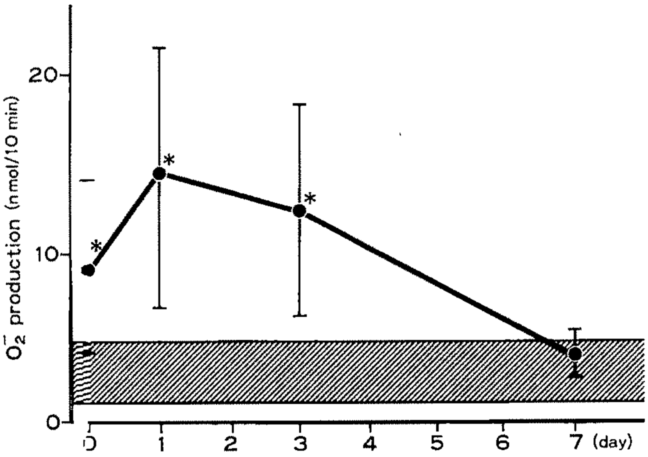

FIG. 3. Serial changes in O_2^- production by neutrophils from 20 trauma patients incubated in buffer alone. Healthy control levels are represented by the shaded area. *p < 0.01 as compared to healthy controls.

TABLE 4. *Serial Changes in GE Secretion by Trauma Patient Neutrophils*

	Control	Day 0	Day 1	Day 3	Day 7
Buffer	5.4 ± 3.7	4.1 ± 2.9	6.6 ± 3.2	4.9 ± 2.4	4.7 ± 3.0
TNF	22.8 ± 7.1*	19.3 ± 9.4*	20.6 ± 10.1*	24.7 ± 11.2*	18.7 ± 10.3*
IL-1α	6.7 ± 4.0	5.7 ± 4.3	5.5 ± 3.1	6.7 ± 4.8	5.3 ± 4.0
LPS	5.7 ± 2.8	5.8 ± 3.6	6.3 ± 3.3	7.2 ± 4.6	5.8 ± 3.8

Serial changes in GE secretion by neutrophils in 20 trauma patients incubated in: buffer; TNF, 10^{-8} mol/L; IL-1α, 10^{-8} mol/L; and LPS, 10 μg/mL. Values are expressed as the mean ± SD.

* $p < 0.01$ as compared to incubation in buffer alone.

responses.[21,22] These results suggest that TNF contributes to stimulation of neutrophil O_2^- production and GE secretion in inflammatory responses.

Recombinant TNF is thought to increase neutrophil adherence, cause neutrophil accumulation, produce superoxide anion, activate neutrophil phagocytosis, and induce release of specific granules.[23] Klebanoff et al.[7] suggest that TNF weakly but directly stimulates the respiratory bursts and lysozome degranulation in neutrophils. The mechanism by which TNF affects neutrophils is not well understood. It is possible that TNF induces changes in neutrophil membranes that trigger the stimulation of oxidative metabolism and O_2^- secretion.[24] Shalaby[15] demonstrated by Scatchard analysis of competitive binding assays that there were approximately 6000 TNF receptors per cell having a molecular weight of 1.37 nmol/L. Neutrophils express a single class of specific high-affinity receptors that are internalized and degraded after TNF binding. The actions of TNF on neutrophils may involve these surface receptors.

Superoxide and GE in blood or tissues have been compared to a two-edged sword in the inflammatory responses. On one hand they assist in clearing cellular debris, destroying toxins, and attacking infectious agents. Thus they can be involved in the pathophysiology of host autoinjury. Superoxide and its metabolites degrade endothelial cells[25] and caused microvascular injury. Granulocyte elastase also lyses a wide variety of normal tissue substrates, such as clotting factors, fibronectin, and tissue components. In healthy individuals GE is regulated by extracellular inhibitors such as α1-PI.[6] However neutrophils, when stimulated, release oxygen radicals that create an oxidative milieu within which α1-PI is inactive. We noted previously that α1-PI concentration increased and controlled GE activity in the blood of trauma patients.[6] *In vitro*, however, after preincubation with TNF, neutrophils produce superoxide that reduces α1-PI activity and disinhibits GE activity (unpublished data).

In conclusion increased neutrophil O_2^- production is a characteristic alteration in the early phase of trauma. Superoxide production and GE secretion in the early phase of trauma may assist host defense. In addition TNF may mediate these effects by provoking O_2^- and GE release from neutrophils involved in the inflammatory response. Further study is required to clarify the mechanisms of O_2^-- and GE-mediated host defense and tissue injury.

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Book Reviews

William P. Longmire, Jr., M.D.
Advisor for Editorial Board
Los Angeles, California

H. Kim Lyerly, M.D.
Advisor for Residents in Surgery
Durham, North Carolina

Wanderjahr: The Education of a Surgeon. Edward D. Churchill (Edited and Annotated by J. Gordon Scannell). 213 pp. Canton, MA: Watson Publishing International, 1990. $18.95.

FOR THIRTY YEARS Edward D. Churchill was the John Homans Professor of Surgery at the Harvard Medical School and Chief of the Surgical Services at the Massachusetts General Hospital (1931–1961). Possessed of a keen mind and unique abilities as a surgeon, investigator and teacher, he created a school of surgeons who hold many important posts in academic and clinical surgery in all parts of the world. One of his students, J. Gordon Scannell, who worked closely with him clinically and in research and has long been a distinguished member of the faculty at Harvard and the Chief of the Thoracic Surgical Service at the Massachusetts General Hospital for many years, has edited and annotated this account of his Chief during the period when Churchill was the Moseley Traveling Fellow in 1926 and 1927. These accounts are based on a series of personal interviews that Dr. Churchill held with Saul Benison, who had been stimulated to undertake this task by the late Isidor S. Ravdin, the John Rhea Barton Professor of Surgery at the University of Pennsylvania. In suggesting this idea, Ravdin thought it essential that such a pioneer as Churchill should be carefully interviewed as a part of the Columbia Oral History Archives. In discussing this challenge with Saul Benison, Ravdin said, somewhat mischievously, "You will find that he is very different from me!"

Dr. Churchill's own commitment in placing emphasis on surgical history is well known, not only to his students but to a host of others who quickly perceived that many of his lectures and clinics began with incisive historical accounts. Furthermore he wrote brilliantly in the field, as evidenced by the scholarly example entitled "To Work in the Vineyard of Surgery."

The introduction to this fascinating travelogue is an account of the life of Churchill as described by Gordon Scannell. It begins with his birth in Chenoa, Illinois in 1895, extends through his early life on the prairie, and then to Northwestern University, where he remained to earn a Masters degree in biology. The latter qualified him to enter the second-year class of Harvard Medical School in 1917, and from that time forward his remarkable career forged ahead. Trained at the famed Massachusetts General Hospital, his brilliance and host of abilities were quickly recognized by his teachers and students alike.

Churchill's close friend at Harvard, William Castle, a research fellow who worked in Cecil Drinker's Laboratory of Physiology, stimulated him to follow an academic career and to engage in original research. Toward this goal he received the Dalton Scholarship from the Massachusetts General Hospital to work in Drinker's laboratory. A devoted scholar, Drinker had been trained by A. N. Richards at the University of Pennsylvania and also by William H. Howell at Johns Hopkins. Walter Cannon invited him to Harvard in 1916, and following World War I, he returned to join the newly created Harvard School of Public Health as Professor of Applied Physiology. It was there that Drinker introduced Churchill to research and where he made firm commitments to research and to prepare for a teaching career.

About this time he received the support of Drinker and of Edsall, the Dean of the Harvard Medical School, for the Moseley Traveling Fellowship. Churchill planned a journey abroad to determine for himself the status of thoracic surgery in the British Isles and Europe at that time.

Throughout the introduction the reader feels the keen appreciation, respect, and affection that Gordon Scannell had for his Chief. Those familiar with Churchill's professional and personal relationships recognized that he always had the greatest admiration for his pupils and avidly supported them in their clinical and academic endeavors. This opening section of this book is beautifully written and contains much information about a mentor, written by one of his brightest students in a very engaging style.

Churchill's recollections of his time in London with visits to Sir Thomas Lewis and others at University College are thoughtfully described. This is followed by a fascinating account of a visit to the Charité in Berlin and the laboratory of Professor Lubarsch, the noted pathologist, and to Otto Warburg's laboratory at the Kaiser Wilhelm Institute to learn the biochemist's method for determining metabolism on small slices of tissue. He then went to the university clinics in Prague and from there to Munich, where he met the famed Sauerbruch as well as his first assistant, Rudolph Nissen, who was subsequently the first to perform a pneumonectomy. Churchill was fascinated by the work of Sauerbruch because he was the world authority in thoracic surgery in that day. Most of his clinical work dealt with thoracoplasty performed as collapse therapy for pulmonary tuberculosis. Churchill's European visit was not all work and included a happy holiday in the Bavarian and Alpine Alps. In this account it becomes obvious that he had a thorough understanding of the countryside and of the people as well.

A visit to Copenhagen brought Churchill to the laboratory of the distinguished physiologist, Krogh, where he worked and was invited to tea on several occasions by the noted investigator himself. In Stockholm he visited Professor Einar Key and admired his technique of thoracoplasty. In Hamburg he saw Professor Ludolf Brauer, a noted pulmonary physician who was unable to find a surgeon specifically to perform the thoracic operations he sought for his patients and therefore became a thoracic surgeon himself. Following the visit to Hamburg, Churchill returned to London and was impressed by Sir Wilfred

Trotter at University College. He was surgeon to King George V and had treated the King's chronic empyema by rib resection with a successful result. He also visited Sir Henry Dale's laboratory, where he had dinner at Emmanuel College, which had been the college of John Harvard while at Oxford.

Perhaps Churchill's career and achievements are best expressed by his closing remarks in his Presidential Address at the American Surgical Association, when he said "In times of change there is need for wisdom both in the external social order and within the profession. Spokesmen who loudly proclaim measures based on self-interest will not be tolerated. A hold-fast in science is essential, but this represents only part of the strength of surgery. By maintaining the ancient bond with humanity itself through charity—the desire to relieve suffering for its own sake—surgery need not fear change if civilization itself survives."

Finally this concise and compelling work holds the reader's attention throughout because it provides a clear understanding of the reasons a committed academic scholar became a surgical master. It can be highly recommended as fascinating and enlightened reading of broad and enduring interest.

DAVID C. SABISTON, JR., M.D.
Durham, North Carolina

Laparoscopic Biliary Surgery. Alfred Cuschieri and George Berci. 101 pp. St. Louis: Mosby-Year Book, Inc., 1990. $95.99

THE BOOK BY CUSCHIERI AND BERCI, *Laparoscopic Biliary Surgery*, is well organized, readable, and informative. The text is not intended to be an encyclopedic description of laparoscopic surgical techniques but rather accomplishes the goal of providing basic information on a relatively new technology.

The first chapter is introductory and refers to Roeder's knots, which are not referred to again until Chapter 3, leaving the reader somewhat mystified by what a Roeder's knot might be. Chapter 2 details the technology of the television-monitoring equipment and provides more information than most surgeons would require. The next several chapters address the technique of laparoscopic examination of the abdomen, cholecystectomy, and cholangiography. More attention could be given to the open technique for introducing the first trocar in the process of laparoscopy because many patients, with previous abdominal operations, are candidates for this technique, and it is safer than the blind technique of trocar introduction. Nitrous oxide may result in the distention of the bowel, particularly in long cases, and is not an anesthetic in a laparoscopic procedure that will last more than 1 hour.

The description of cholangiography is quite well done, and the description of the cholecystectomy procedure itself is effective, clear, and appropriate. The authors suggests that if the gallbladder is accidently opened during the course of its removal, a full course of antibiotics should be given. There is very little defense for this argument, and no data to support it. They also suggest that acute cholecystitis is a contraindication for the laparoscopic removal of the gallbladder, which is absolutely not the case; we have been able to remove acutely inflamed gallbladders fairly regularly.

The suggestion that injury to the common bile duct is a consequence of poor patient selection is misleading. Injury to the common bile duct in laparoscopic cholecystectomy, as with open cholecystectomy, is a consequence of surgical technique and judgment and has little to do with patient selection. It is now clear that the common duct injury incidence is three times that of open cholecystectomy. This incidence of injury to the common bile duct is changing rapidly with experience but has been a major problem.

The chapter devoted to laparoscopic cholecystostomy is interesting, but the technique will probably find infrequent application.

The book is well written, organized, and illustrated and will provide useful information to the surgeon beginning laparoscopic cholecystectomy.

LARRY C. CAREY, M.D.
Tampa, Florida

Complications in Cardiothoracic Surgery. John A. Waldhausen and Mark B. Orringer. 460 pp. East Norwalk, CT: Mosby-Year Book, 1991. $110.00

THIS TEXTBOOK, DEVOTED to the description and management of complications following thoracic and cardiac surgical procedures and the perioperative care of such patients, fills a unique niche in the cardiovascular surgical literature. Such a broad and in-depth compilation of this information, by editors Waldhausen and Orringer, had not yet been achieved in this surgical subspeciality before this edition.

There are four parts to the text. Part I, containing 14 chapters, deals with the management of complications related to the perioperative care of cardiothoracic surgical patients and ranges from anesthetic complications to management of complications arising from pacemaker insertion. Part II deals with the management of complications of open and closed cardiac surgical procedures. In these 16 chapters the major congenital lesions are discussed, including aortic arch and pulmonary artery anomalies, septal defects, tetralogy of Fallot, venous drainage anomalies, arterial and atrial switch procedures for transposition, and the Fontan operation. Also included in this section of the book are discussions of the complications that occur after adult cardiothoracic surgical procedures, including aortic aneurysms, valvular heart disease, ischemic coronary artery disease, and supraventricular and ventricular arrhythmias. Part III, comprised of eight chapters, covers the complications following general thoracic surgical procedures, including endoscopy, pulmonary and esophageal resection procedures, hiatal herniorrhaphy, thymectomy, motility disorders, and mediastinal and chest wall tumors. Part IV discusses complications that occur after heart and heart-lung transplantation.

As stated in the preface to the book, the goal of the editors was to encompass the entire field of cardiothoracic surgery; this has certainly been achieved, with only lung transplantation and several rare forms of congenital heart disease not treated in depth. The 86 authors, all experts in their respective areas, have drawn on their own experience as well as that of others in presenting their information, such that the discussion of the complications and their treatment is truly "state of the art." In each chapter the complications are discussed objectively and the etiologic factors that caused the complications and the options for treatment are approached as scientifically as possible. Multiple tables presenting compiled data and figures illustrating surgical techniques punctuate the text.

This outstanding textbook represents a superb addition to the existing literature in cardiothoracic surgery. The completeness of the text, the objectivity in the presentation and discussion of the complications, and the effectiveness of the options for treatment make this a necessary addition to the library of cardiothoracic surgical residents and practicing cardiothoracic surgeons alike.

T. BRUCE FERGUSON, JR., M.D.
St. Louis, Missouri

Multiple Organ Failure: Patient Care and Prevention. Arthur E. Baue. 561 pp. St. Louis: Mosby-Year Book, Inc., 1990. $79.00

MULTIPLE-ORGAN FAILURE has reached epidemic proportions in most intensive care units and is fast becoming the most common cause of death in surgical patients. Thus the recent publication of this monograph, as well as two other texts on the subject of multiple-organ failure, is long overdue. One major difference between this book and the others published on this topic is that Dr. Baue's work is single authored while the others are multiauthored. Because of the cross-disciplinary nature and complexity of the subject matter, before reading this text, I was concerned that, despite Dr. Baue's unique and excellent qualifications, some of the topics covered in the text would be limited in scope, depth, or sophistication. I am happy to say that this fear was largely unfounded. Although not everyone will accept all the principles espoused by the author, and some will question some of the author's biologic conclusions, or even be willing to debate the ultimate accuracy of the authors theoretical constructs, no one will argue over the immense value of this book. In an area, such as multiple-organ failure, where our level of knowledge is incomplete and where an enormous amount of new and often contradictory information is generated almost daily, it is important that we take stock of our standing in the field. This Dr. Baue does well. Throughout the text he clearly distinguishes between the established, the suspected, the controversial, and the unknown. This approach will allow the reader to acquire a foundation of knowledge that is built on established clinical and physiologic principles rather than speculation or half truths. Of equal importance, Dr. Baue has identified clearly key basic science and clinical hurdles that must be crossed to improve the clinical outcome in patients with organ failure. Thus, although this text is one man's odyssey through difficult terrain, the trip is worth the taking.

The book consists of 31 chapters and is divided into six sections. Section one deals with the biologic response to injury and is exceptionally well done. The chapters in this section provide much of the biologic underpinning for the remainder of the book. Section two is concerned with the prevention of complications, while section three addresses trauma. The last three sections deal specifically with multiple-organ failure and cover the topic at both the individual organ (section 4) and systems levels (sections 5 and 6). Due to the inclusive nature of the material covered in the text, I recommend it to all those who have an interest in the subject, from the first-year house officer to the most experience and sophisticated clinician or investigator.

EDWIN A. DEITCH, M.D.
Shreveport, Louisiana

Atlas of Abdominal Surgery. John W. Braasch, Cornelius E. Sedgwick, Malcolm C. Veidenheimer, and F. Henry Ellis, Jr. 464 pp. Philadelphia: WB Saunders, 1991. $95.00.

THIS ATLAS FOCUSES on the techniques of the common and not so common procedures performed by general and gastrointestinal surgeons. It consists of 464 pages, is very well illustrated, and provides a genuine resource for the practicing surgeon. The book is divided into two large sections: the gastrointestinal tract; and the liver, biliary tract, pancreas, spleen, adrenal glands, and abdominal wall hernias. These areas are covered in a total of 59 chapters. Fourteen authors have been included, all of whom are prominent in their respective fields. If there is any flaw in this atlas, it is perhaps that the techniques described may reflect the practice at a single institution and may not be representative of how these various operations are performed in other parts of the country. Nonetheless the style of the atlas is such that all surgeons can glean important information from this work and include hints and tips in their daily practice.

The editors have structured the individual chapters to focus on operations as well as disease entities. This is noted, for example, in the biliary section, where in one chapter choledochoduodenostomy is described. In a subsequent chapter the authors focus on the management of choledochal cysts, and in a subsequent chapter on proximal bile duct tumors. In general the work is current. This is reflected by the inclusion of sections dealing with bariatric surgery, hepatic transplantation, and laparoscopic cholecystectomy. The details of the various procedures discussed are not as extensive as has been provided in other atlases. This is probably intentional because this work is really targeted for the practicing surgeon. Each operation is detailed and a summary of both the important features and steps or plans is provided. This is a very helpful style, particularly for the surgeon who is already acquainted with these operations and is merely looking to refresh his or her memory. The description of the procedures are clear and concise. The illustrations are of high quality and contribute to the overall readability of this work.

The editors intended to provide a work that would focus on the art of surgery, as opposed to its science. They specifically do not address diagnostic evaluation or pre- and postoperative care. I think this is intentional to separate technique from science and they have been enormously successful in their endeavors. This is a well-done, valuable atlas that provides helpful information for the practicing surgeon who performs gastrointestinal and other abdominal procedures.

JOEL J. ROSLYN, M.D.
Los Angeles, California

Current Critical Problems in Vascular Surgery (Vol.2). Frank J. Veith. 475 pp. St. Louis: Quality Medical Publishing, 1990. $79.95.

THE SECOND VOLUME of *Current Critical Problems in Vascular Surgery* is an account of topics presented at the 1989 Montefiore-Einstein Symposium on Current Critical Problems and New Horizons in Vascular Surgery. What has made the symposium a success is the outstanding faculty and the unique approach to the topics selected. These same elements are the key ingredients to the success of the book. The list of contributors includes many distinguished vascular physicians. In the selection of topics, Dr. Veith was not hampered by many of the constraints facing the editors of the major vascular textbooks. By the nature of the symposium, the book's audience is familiar with vascular disease and only needs additional knowledge to strengthen this foundation. The editing of the book reflects this, devoting very little text, other than introductory paragraphs, to background material.

For the common vascular procedures, the book describes innovative techniques that are applicable to routine clinical practice. For lower-extremity bypass, the major conflicting viewpoints, with their respective supporting data, are presented in the autogenous *versus* prosthetic graft argument. The section on carotid surgery portrays this controversial subject in an interesting fashion. The first chapter is by Dr. Imparato and the closing chapter is by Dr. Barnett, who both combine data with philosophy to stimulate thought on this important topic.

Chapters on the various percutaneous methods of treating vascular disease are well presented. The research directions given throughout the book are written by the pioneers in the respective fields and cover the important avenues for the future.

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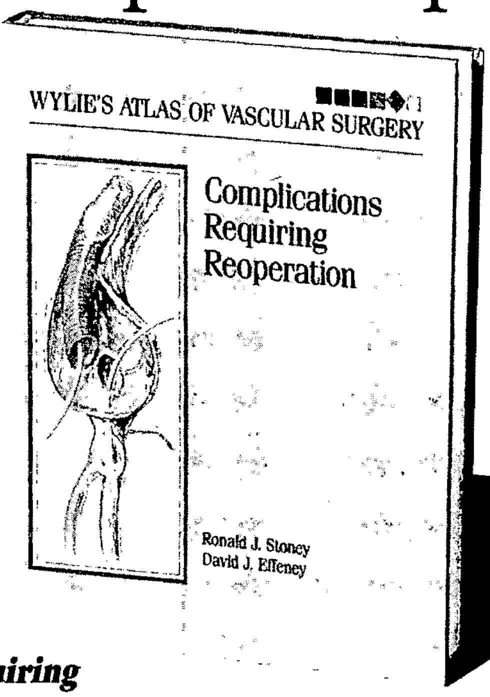
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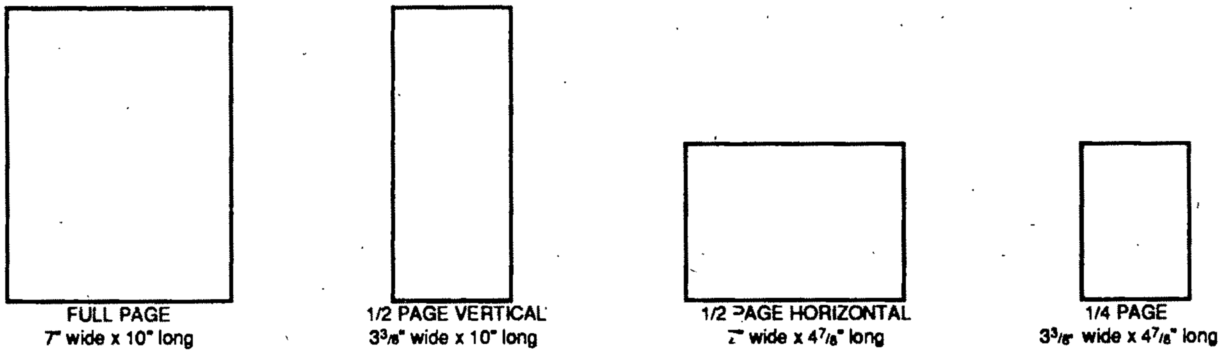

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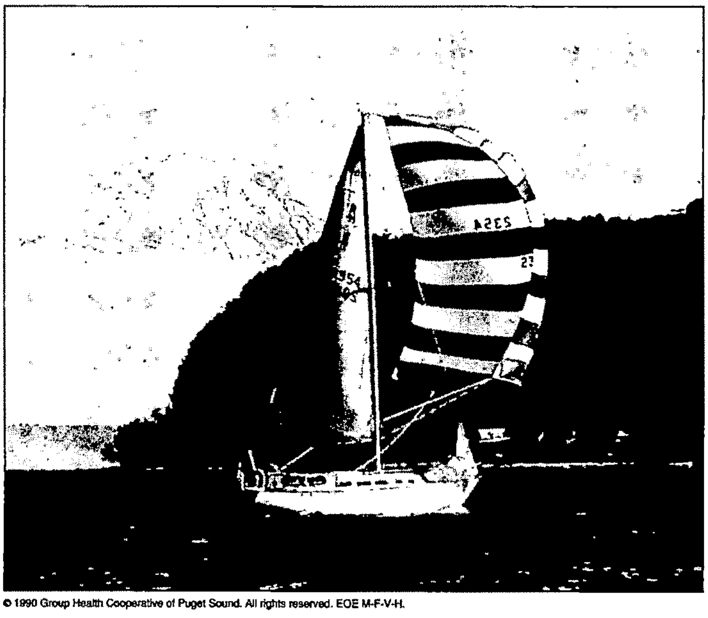

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The Right Thing

ROBERT ZEPPA, M.D.

In his recent and masterful Shattuck Lecture, "Healing and Heroism," Brownell Wheeler,[1] through the artifice of Osler's perhaps "perspective of the contemporary medical scene," concluded that Sir William "would be aware of a deep seated malaise." This is indeed a most serious diagnosis and implies the presence of an illness that could be threatening to the fabric of our profession. The question, of course, concerns the accuracy of this diagnosis If it is accurate, what is the remedy?

I agree with Dr. Wheeler, both from personal experience, some of which I shall relate, and from other recent writings of our colleagues, which I shall cite, that there is indeed angst in the profession.

A few years ago I was asked by the attorneys representing the American College of Surgeons to participate in the preparation of some of the evidence pertaining to a case of itinerant surgery. As you may recall, the College was being sued by a surgeon who had been dropped from the rolls because of his participation in this nefarious activity. My role in this matter was to review dozens and dozens of charts from several hospitals and to abstract the surgeon's participation in the care of patients. The appalling truths revealed in those documents was that a surgeon relinquished all of the preoperative and postoperative care of these patients to physicians who were not surgeons. In fact, often the first time the patient was seen by the surgeon was on the operating table and asleep, and just as often was never seen again, whether alive or dead. I can still recall the opprobrium that this experience engendered. How was informed consent obtained? What was the surgeon–patient relationship under these circumstances? Obviously there were none! Peabody's dictum concerning caring for the patient was notable only in its absence. Such doings serve to degrade our profession to less than a craft and a craft may be identified as the application of dexterity as in manual employment. Certainly such self-serving activity does not conform to even Webster's[2] definition of a profession: "A profession is a calling, requiring specialized knowledge, often long and intensive preparation including instruction in skills and methods as well as in the scientific, historical or scholarly principles underlying such skills and methods, maintaining by force of organization or concerted opinion high standards of achievement and *conduct* and committing its members to continued study and to a kind of work which has for its prime purpose the rendering of *public service*." Was this activity merely an instance of aberrant behavior or did it signify, as Wheeler has written, "a deep seated malaise"? This was so gross a defilement of appropriate surgical behavior that at the time I chose to regard it as a singular episode from which no generalizations could be derived.

Yet Wheeler's lecture is replete with illusions to the dearth of humanistic behavior in the practice of medicine today and he is not alone in this opinion. In his presidential address before the New England Surgical Society in 1989, Jim Foster presented "Some Eastern Thoughts for Northeastern Surgeons"[3] in which he stressed "that values and attitudes are more important than measurements and prices." He lamented the inroads made by "market amorality" into the practice of our profession. These concerns are similar to those voiced by Wheeler in decrying those initiatives that are "self-serving or financially motivated."

Are these the thoughts of two lonesome prophets in a wilderness that has not been completely charted? Are they akin to the deductions of the blind man attempting to describe an elephant as a tree trunk or a snake? I think not!

In his Scudder Oration on trauma presented at the American College of Surgeons Clinical Congress in 1989,

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Presented at the 111th Annual Meeting of the American Surgical Association, April 11–13, 1991, Boca Raton, Florida.

Address reprint requests to Dr. Robert Zeppa, Department of Surgery, University of Miami School of Medicine, P. O. Box 016310, Miami, FL 33101.

Accepted for publication April 22, 1991.

Trunkey asked "What's wrong with trauma care?"[4] He identified issues germane to his answers. One was "abdication of leadership," which he lay at the feet of surgeons and hospital administrators. After reviewing the evidence for the efficacy of trauma systems in saving lives and rehabilitating the injured, he impugned surgeons and administrators alike for failure to provide the leadership for the development of such systems. Lack of so-called appropriate reimbursement was seen as an important factor in this and he called for some "fair proportion of charity work." In his "damn the torpedoes" style, Trunkey identified reasons that he considered operational for surgeons' lack of commitment to trauma care. One, the "hunger for more" syndrome, which, of course, freely translates to greed. Furthermore he rightly identified trauma as a "nocturnal disease" and pointed out that it can be "destructive to social life and to elective surgery practice." He noted that "many of the patients are perceived as being lower class, indigent, and disrupting to a hospital or office practice. Are these harsh judgments based on insufficient data? Let me recount yet another anecdote.

In 1982 a young faculty member, whom I had appointed to head our trauma service (the late David Kreis), conducted a preventable death study for Dade County using the methods described by West and Trunkey. While the numbers were not as bad as those identified in Orange County, California, still they served as the impetus to the establishment of a county-wide trauma system. The negotiations were frequently acrimonious and pecuniary motivations clashed, but at long last the system came into being on October 1, 1985. Dade County had an advantage in that a well-established and extremely competent Fire Rescue Network was in place throughout the county, and before implementation to the system, the county bought a rescue helicopter. We began with eight hospitals in the system; there was one level one, six level two, and one pediatric facility. The triage criteria used were those published by the Committee on Trauma of the American College of Surgeons. During that first year of operation there were more than 9000 trauma transports and more than 60% of the victims were discharged from the trauma centers. However the honeymoon was about over and on or about November 1, 1986, groups of neurosurgeons, orthopedic and thoracic surgeons announced publicly that they would no longer take trauma call because of the malpractice problem. Our own inquiries failed to reveal an increase in the rate of suits brought by trauma victims, but when a suit did occur the victim was more likely to be indigent. Nothing galls more than a malpractice suit brought by a patient who has not paid the bill.

Despite the appearance of the physicians' announcement in the local press, not a single inquiry related to the ethical or moral questions that might be involved in making such a decision was ever made publicly by (1) the political leaders of the community, (2) representatives of the media, or (3) organized medicine. It didn't seem to matter.

During the year of operation of the system with the eight hospitals, Jackson Memorial received approximately one third of the patients transported. By December 1986, 1 month after the physicians' public announcement, more than 60% of the injured people in the county were brought to this hospital. By the following July 1987, all of the hospitals except Jackson Memorial and Miami Childrens had designated themselves as trauma centers. Since then virtually all of the adult trauma victims and approximately 90% of the pediatric injuries in this county of 2 million people and 2000 square miles are treated in a single facility.

Do I think in company with the three colleagues cited above that a serious disease has afflicted our traditional value systems in medicine? You can bet your life that I do! What ever happened to the concept of tithing in the public interest? Unfortunately instead of providing role models for the society, we physicians increasingly display the same "market place amorality." Chris Black,[5] on the staff of the *Boston Globe*, has noted that selfishness has replaced community responsibility in our country.

Further in his recent essay on the human condition, Razis[6] described Koestler's observations concerning two fundamentally different curves that may describe some of human history. The accumulation of knowledge, communications power, and population is a curve that rises exponentially. Unfortunately the curve describing the growth of ethics, social moralities, spiritual awareness, and related values is nearly flat. As for the physicians, Uwe E. Rheinhardt has written "the individual American physician finds himself or herself today reduced somewhat in stature, though not in wealth, almost a mere peer among an ever increasing number of profit oriented purveyors of health care, each competing vigorously for the health care dollar."[7]

While I agree with Foster that "moralizing is always easier on the preacher than the preachee" yet, if you believe there is a kernel of truth in this polemic, we must seek a remedy. Surgery, the crown jewel in the tiara of medicine, devotes a minimum of 5 years to the education of its disciples and for the most part we evaluate the efficacy of this educational experience in numbers. The number of colonoscopies, the number of femoropopliteal bypasses, and many other such numbers form the basis for the approval or disapproval of programs. One might ask if this provides reasonable guidelines for evaluating our educational endeavor, and, of course, the answer is yes. Surgery is not only an art that may be defined as the general principles of any branch of learning but also involves the craft of surgery. My thesis is that we have come to stress too much the craft of surgery and have, to a large degree, avoided evaluating the art of surgery in our residents. Remember that it has been the application of the art of medicine that earned physicians a respected and prominent place in the hearts of men and women.

Since we have no tests or measurements to gauge the healers' art, we must rely on education. While numbers provide some assurance that the craft of surgery will be imparted, yet we also evaluate the educational experience in units of time. So many months for this experience or that are part of the requirements for the approval of training programs. Why don't we require, in our training programs, a specified amount of time for the study of ethics and for solving moral dilemmas?

Certainly many medical schools have introduced ethics into their curricula as a formal and important study. Yet we have no prescribed mechanism for the continuation of such studies in our postgraduate educational programs. I am sure you will agree that as the technology of medicine becomes increasingly complex and even hazardous, the ethical dilemmas grow apace. Such problems are aired almost daily in the press. It is my contention that the American Board of Surgery and the Residency Review Committee for Surgery should borrow the concept of ethical training from Rollo Hanlen's experience in the American College of Surgeons and expand it. Surely in 5 years of training, 50 or 100 hours of ethics could be introduced without strain. Whatever the number of hours, the experience should be mandatory. Many major hospitals in this country have ethicists on staff and for those who do not, hospital chaplains abound.

I grant that we will probably not have means to judge the efficacy of such an experience; however we will have the satisfaction of knowing that our residents have been introduced to ethical thought and have participated in the discussion of moral dilemmas. At least the experience should provide them with the appropriate foundations for one of the most difficult of human endeavors—doing the right thing.

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The Optimal Extent of Resection for Patients With Stages I or II Breast Cancer Treated With Conservative Surgery and Radiotherapy

FRANK A. VICINI, M.D.,* TIMOTHY J. EBERLEIN, M.D.,† JAMES L. CONNOLLY, M.D.,‡ ABRAM RECHT, M.D.,* ANTHONY ABNER, M.D.,* STUART J. SCHNITT, M.D.,‡ WILLIAM SILEN, M.D.,§ and JAY R. HARRIS, M.D.*

From the Department of Radiation Therapy, Harvard Medical School, and Joint Center for Radiation Therapy, Department of Surgery,† Brigham and Women's Hospital and Harvard Medical School, and Departments of Pathology,‡ and Surgery,§ Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts*

The optimal extent of breast resection before irradiation for treatment of early breast cancer has not been defined. Increasing the size of the resection may decrease the risk of local recurrence but will also have an adverse impact on the cosmetic outcome. The 5-year likelihood of a recurrence of the tumor was analyzed in relation to the volume of resected breast tissue in 507 patients with infiltrating ductal carcinoma treated with conservative surgery and radiation therapy between 1968 and 1982. Patients were stratified by clinical T-stage and for each T-stage patients were divided into three groups of equal numbers based on the volume of excised tissue. All patients had at least a gross excision of the tumor and the extent of breast resection was determined at the discretion of the surgeon without knowledge of the histologic features of the tumor. The median follow-up time was 100 months. The 5-year actuarial recurrence rates were analyzed in relation to clinical T-stage (T_1 or T_2) and the presence or absence of an extensive intraductal component (EIC+ or EIC−). For patients with EIC+ tumors, the largest resections were associated with a substantially lower risk of recurrence in the breast than the smallest resections. This effect was seen both for T_1 tumors (10% *versus* 29%, $p = 0.07$) and for T_2 tumors (9% *versus* 36%, $p = 0.04$). For patients with EIC− tumors, recurrence rates were significantly lower than for EIC+ tumors and were not influenced by the volume of resection to the same degree as EIC+ tumors. In the absence of an EIC, recurrence rates for the largest and smallest resections were 0% and 9% ($p = 0.02$) for T_1 tumors and 3% and 6% (p = NS) for T_2 tumors. It is concluded that a limited breast resection is acceptable for an EIC− tumor but that a more extensive resection is required for an EIC+ tumor. These results stress the importance of assessing the presence or absence of an EIC in determining the optimal extent of breast resection required before radiation therapy.

As THE USE of conservative surgery (CS) and radiation therapy (RT) has become an accepted option in the treatment of early-stage breast cancer,[1] the issue of the optimal extent of breast resection before RT has assumed greater importance. Some studies have shown that increasing the size of the breast resection may diminish the risk of local recurrence,[2] but others have shown that this will also have an adverse impact on cosmetic outcome.[3,4] Assessing the microscopic margins of a 'lumpectomy' specimen would appear to be the most logical means of ensuring adequate tumor removal before RT without unnecessarily worsening cosmetic outcome in most patients. Unfortunately what constitutes an 'adequate' margin is undefined[5,6] and the impact of involvement of microscopic margins on the risk of local recurrence after CS and RT has not been established.[2,7-10]

We have shown previously that assessing infiltrating ductal cancers for the presence or absence of an extensive intraductal component (EIC) can be helpful in judging the adequacy of tumor removal after local excision.[11] In a study of patients who underwent a re-excision of the primary tumor after an initial gross excision because of positive or close margins of resection, we found that patients with EIC+ tumors had a greater incidence of residual tumor (frequently widespread) in the re-excision specimen than did patients with EIC− tumors (88% *versus* 44%, $p = 0.002$). In the present report, we address the issue of the volume of resected breast tissue, its relation to the presence or absence of an EIC, and their combined effect on the likelihood of a local recurrence of the tumor. Our data suggest that a limited breast resection is acceptable for an EIC− tumor but that a more extensive breast resection is required for an EIC+ tumor.

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Address reprint requests to William Silen, M.D., Department of Surgery, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215.

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Methods

From 1968 to 1982, 954 women with unilateral Union Internationale Contre le Cancer/American Joint Committee for Cancer Staging and End-Results Reporting (UICC-AJCC) clinical stage I or II breast cancer were treated at the Joint Center for Radiation Therapy in Boston, Massachusetts. To eliminate the confounding effects of inadequate treatment, 29 patients with less than an excisional biopsy and 116 patients who received a total dose of less than 60 Gy to the region of the primary tumor were excluded from the analysis, leaving 809 clinically eligible patients. Patients with simultaneous bilateral tumors were excluded and, for patients with metachronous opposite breast cancer, only the first tumor was considered for the analysis of failure.

Initial biopsy material was reviewed by two of the authors (JL Connolly and SJ Schnitt) for 736 breast tumors (91%). Details of this assessment have been previously described.[12] For this report, analysis was limited to 507 of these 736 cases, in which the histologic type was infiltrating ductal carcinoma, sufficient grossly normal adjacent breast tissue was present for evaluation, and the total volume of excision could be calculated. Pathologic examination assessed the presence and extent of intraductal carcinoma both in the tumor mass and in the adjacent grossly normal breast tissue.

As of December 1989, median follow-up time was 100 months for the 366 surviving patients (range, 17 to 219 months). The median follow-up time for all patients was 93 months (range, 5 to 219 months). Six patients were lost to follow-up 17 to 78 months after treatment.

The details of treatment have been previously described.[13] Surgery consisted of excisional biopsy, defined as gross total removal of the primary tumor, usually without histologic confirmation of pathologic margins. A total of 399 patients (79%) underwent axillary dissection, usually limited to levels I and II.

All patients received whole breast irradiation, typically to a total dose of 45 to 50 Gy during a period of 5 weeks. This was followed by a boost to the original tumor site to at least 60 Gy using either an interstitial implant (Iridium-192), photons, and/or electrons.

Chemotherapy was administered to 125 patients (25%) in various sequencing with radiotherapy. One hundred fourteen of one hundred forty-two patients (80%) with histologically positive axillary lymph nodes received adjuvant chemotherapy. Only 3% (7 of 257) of patients with negative axillary nodes received chemotherapy. The chemotherapy regimens varied substantially during this period, but 90% of patients receiving chemotherapy were treated with cyclosphosphamide, methotrexate, and 5-fluorouracil (CMF) or with doxorubicin.

Our previous experience has shown that the most important predictor of recurrence in the breast is what we have termed an extensive intraductal component (EIC) in the primary excision specimen. This is defined as the simultaneous presence of both a prominent amount of intraductal carcinoma within the primary tumor and intraductal carcinoma clearly extending beyond the infiltrating margin of the tumor or present in grossly normal adjacent breast tissue. It may also denote a tumor that is predominantly noninvasive with focal areas of invasion.

The gross volume of excised breast tissue was calculated by multiplying height × width × length, using maximum dimensions of the gross specimen as described by the pathologist. To determine the impact of the extent of breast resection on the risk of recurrence in the breast, the 265 patients with T_1 tumors were divided into three groups of equal numbers based on the volume of breast tissue resected (less than 13 cm^3, 13 to 48 cm^3, and more than 48 cm^3). The 242 patients with T_2 tumors were similarly divided into three groups of equal numbers with volumes less than 35 cm^3, 35 to 74 cm^3, and more than 74 cm^3.

Recurrence in the breast ('local recurrence') was defined as the detection of cancer in the parenchyma and/or skin of the treated breast occurring before or simultaneously with the discovery of distant metastases. Recurrences were classified by the location in the breast relative to the primary tumor and boost volume as true recurrence (TR, within the area of the boost), marginal miss (MM, adjacent to the boost), elsewhere (several centimeters from the primary site), and other (skin only and unclassifiable). Because we consider TRs and MMs to be recurrences of the primary tumor (as opposed to a new primary in the ipsilateral breast), only TRs and MMs were used in the calculation of local failure.

Failure rates were calculated using two methods: crude 5-year percentages and actuarial curves (by the Kaplan–Meier method).[14] Patients were censored from the calculation of local recurrence at the time of last follow-up examination, the discovery of metastatic disease, the discovery of regional disease without local recurrence, or death from intercurrent illness. Six patients lost to follow-up were all without evidence of recurrence and were deleted from calculations at last known follow-up. Patients were censored from calculations of distant failure at the time of death from intercurrent illness or at last follow-up. Distant failure was scored at the time of first discovery of disease outside of the treatment field (but excluding isolated nodal failure). Cause-specific survival rates were calculated by scoring death from breast cancer and its sequelae, deleting patients who died of other causes at the time of death and those lost to follow-up at last known follow-up. All intervals were calculated from the date of beginning radiotherapy. The statistical significance of the differences between actuarial curves was calculated using the two-tailed log-rank test. Probability values of 0.05 or less were considered statistically significant.

Results

Fifty-five of the five hundred seven patients (11%) developed local recurrence of the tumor in the treated breast. Forty of these recurrences occurred within the first 5 years after treatment, for a crude 5-year rate of 8%. The 5- and 10-year actuarial rates of local recurrence were 12% and 17%, respectively, and the 5- and 10-year actuarial rates of distant failure were 21% and 31%, respectively. Actuarial survival rates were 86% at 5 years and 75% at 10 years, with 80 patients surviving for more than 10 years.

Table 1 shows the association between the extent of breast resection and the 5-year actuarial rate of recurrence in the breast. Overall patients with EIC+ tumors had higher rates of recurrence than patients with EIC− tumors. For patients with clinical stage T_1 EIC+ tumors, the recurrence rate was 29% for those with resection volumes less than 13 cm^3, 22% for those with resection volumes between 13 and 48 cm^3, and 10% for those with resection volumes greater than 48 cm^3 (29% *versus* 10%, $p = 0.07$). For patients with clinical stage T_2 EIC+ tumors, the recurrence rate was 36% for those with resection volumes less than 35 cm^3, 26% for those with resection volumes between 35 and 74 cm^3, and 9% for those with resection volumes greater than 74 cm^3. The difference between recurrence rates for patients with the smallest and largest breast resections was statistically significant (36% *versus* 9%, $p = 0.04$). The recurrence rates were not significantly different between the intermediate and largest resection groups for patients with T_1 tumors and for patients with T_2 tumors.

In patients with EIC− tumors, low rates of local recurrence were obtained for all subgroups. For patients with clinical stage T_1 EIC− tumors, the recurrence rate was 9% for those with breast resection volumes less than 13 cm^3, 2% for those with resection volumes between 13 and 48 cm^3, and 0% for those with resection volumes more than 48 cm^3 (9% *versus* 0%, $p = 0.02$). For patients with clinical stage T_2 EIC− tumors, the recurrence rate was 6% for those with breast resection volumes less than 35 cm^3, 2% for those with resection volumes between 35 and 74 cm^3, and 3% for those with resection volumes greater than 74 cm^3. The differences between these recurrence rates were not statistically significant.

TABLE 1. *Five-year Actuarial Rate of Recurrence of the Tumor in the Breast in Relation to the Extent of Breast Resection and the Presence of EIC*

	Tumor Size	No. of Patients	Extent of Breast Resection*			Probability Value†
			Smallest	Intermediate	Largest	
EIC^+	T_1	83	29%	22%	10%	0.07
	T_2	78	36%	26%	9%	0.04
EIC^-	T_1	182	9%	2%	0%	0.02
	T_2	164	6%	2%	3%	NS

* For patients with T_1 tumors, the volume of breast resected corresponds to <13 cm^3, 13–48 cm^3, and >48 cm^3 for smallest, intermediate, and largest resections, respectively.

For patients with T_2 tumors, the volume of breast resected corresponds to <35 cm^3, 35–74 cm^3, and >74 cm^3 for smallest, intermediate, and largest resections, respectively.

† Probability values compare differences between the largest and smallest breast resections.

Discussion

In this study we examined the association between the extent of breast resection and the risk of a recurrence of the tumor after CS and RT. We found that in patients with an EIC+ tumor, the largest resections were associated with a significantly lower risk of recurrence in the breast than the smallest, both for T_1 and T_2 tumors. For patients with EIC− tumors, recurrence rates were significantly lower than for EIC+ tumors and were not influenced to the same degree by the volume of resection. Our results suggest that in the absence of an EIC in the primary tumor, a limited excision of the tumor is adequate for patients with stages I and II infiltrating ductal breast cancer. In contrast larger resections are necessary in patients with EIC+ tumors to achieve an acceptable rate of local control.

The optimal extent of breast resection necessary to obtain an acceptable rate of tumor control while maintaining good cosmetic results after CS and RT has been widely debated. A critical and as yet unresolved issue is the determination of when an 'adequate' resection of the tumor has been achieved. One approach has been to assess the microscopic margins of excision of the resected specimen. Unfortunately there are many problems inherent in defining such margins. Breast specimens are fatty and irregular, making assessment of margins difficult, even with the use of ink to denote surfaces. Routine sectioning by the pathologist does not usually permit examination of the entire surface area of the specimen.[6] In addition the intraductal component associated with the tumor has a highly complex, ramifying structure so that in any given section it may not appear continuous. Given these limitations, it has been shown that even in the presence of what appear to be 'negative' margins, approximately one third of breast cancers have additional foci of cancer beyond the limits of the resection.[5,6] In addition the clinical significance of 'focally positive' or even 'prominently positive' microscopic margins is not clearly established in patients treated with CS and RT.[7–10] A reproducible and reliable technique for assessing margins remains to be defined.

The association between the extent of mammary resection and outcome for patients treated with CS and RT has been examined in several large series.[7,15–19] In Table 2 results of excisional biopsy (lumpectomy) are compared with those of quadrantectomy (or wide resection). These

TABLE 2. *Local Recurrence Related to the Extent of Breast Resection*

	Percentage Local Recurrence	
Series	Excisional Biopsy	Quadrantectomy
Retrospective		
Clarke*	5% (369)	9% (43)
Van Limbergen†	15% (149)	7% (69)
Chu	10% (110)	11% (18)
Nobler‡	7% (87)	6% (48)
NSABP§	10% (629)	—
Veronesi‖	—	3% (1232)
Prospective		
Veronesi	7% (345)	2.2% (360)

* Crude rate of local recurrence—median follow-up 60 months.[7]

† Five-year actuarial rate of local recurrence—median follow-up 80 months.[15]

‡ Crude rate of local recurrence—median follow-up 46 months.[16]

§ Eight-year actuarial rate of local recurrence—mean follow-up 81 months.[17]

‖ Ipsilateral recurrence of the tumor—median follow-up 72 months.[19]

retrospective analyses do not establish a consistent advantage for the use of quadrantectomy compared to more limited resections. In 1985,the National Cancer Institute in Milan initiated the only prospective randomized trial comparing 'lumpectomy' followed by RT (including a boost with Iridium-192) with quadrantectomy and RT in patients with primary tumors smaller than 2.5 cm.[2] With 705 cases that could be evaluated, patients treated with lumpectomy had a higher frequency of local recurrence than patients treated with quadrantectomy (7% *versus* 2.2%). Quadrantectomy in this study (as well as in the retrospective series) clearly resulted in an inferior cosmetic outcome, however.

The studies described above do not confirm a consistent advantage for performing larger resections before irradiation for all patients. This in part may be related to the variability in the initial tumor burden in breast cancer patients. As a result some patients may undergo excessive breast resections (with an associated poorer cosmetic result) while others may have inadequate excision of the tumor. Any determination as to the impact of the extent of the surgery on outcome thus becomes difficult to establish. These studies stress the importance of defining a histopathologic or biologic marker that predicts the extent of breast resection required before RT. Previous studies from our institution suggest that the presence of an EIC may be such a marker.[13] Holland et al.[20] performed simulated gross excisions of tumors on mastectomy specimens and then determined the extent and type of residual tumor in the remaining breast tissue. Residual disease was found in the breast in 71% of women with EIC+ tumors compared with 28% of those without an EIC ($p < 0.0001$). Approximately 30% of patients with EIC+ tumors had 'prominent' residual intraductal carcinoma (six or more low-power fields required to encompass the disease) located at least 2 cm beyond the edge of the primary tumor, compared with only 2% of patients with EIC− tumors. In a study of patients who underwent a re-excision of the primary tumor site because of positive or close margins of resection after an initial gross excision, Schnitt et al.[11] similarly found that patients with EIC+ tumors had a greater incidence of 'prominent' residual tumor in the re-excision specimen than did patients with EIC− tumors (44% *versus* 2%, $p < 0.0001$). These data helped to generate the hypothesis that the optimal extent of breast resection can be judged on the basis of the presence or absence of an EIC. The results of the present study lend additional support for this hypothesis.

The data presented here indicate that a limited breast resection is acceptable for an EIC− tumor but that a more extensive resection is required for an EIC+ tumor. It is worthwhile to note that for EIC− tumors, patients with intermediate volume breast resections had an excellent rate of local control, not substantially different from that of patients with the largest resections. These results have ramifications for cosmetic outcome as well as local control because cosmetic outcome after CS and RT is most adversely affected by the extent of resection.[3,4] By assessing the presence or absence of an EIC, the extent of breast resection required before RT can be minimized in the approximately two thirds of all patients with infiltrating ductal carcinomas whose tumors are EIC−, thereby improving the likelihood of a good cosmetic result without decreasing the level of local control. These data also suggest that if patients with EIC+ tumors undergo a wide excision, acceptably low rates of local recurrence can be obtained.

We recognize that there are limitations to the practical usefulness of assessing tumors for the presence of an EIC. As always the reproducibility of the diagnosis by the pathologist needs to be considered (*i.e.*, interobserver variability in applying the definition). In addition determining the presence of an EIC requires a more detailed and time-consuming pathologic assessment than is commonly performed. If the use of pathologic criteria to determine the extent of operation is not deemed feasible, it is likely that either a wide excision will be required in all patients (with poorer cosmetic outcome) or highly restrictive selection criteria will be needed to minimize the risk of recurrence.

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DISCUSSION

PRESIDENT ROBERT ZEPPA: Thank you, Dr. Silen, and before introducing the discussants who have been asked to discuss this paper, I would like to ask one question. Bill, was there any difference in the backgrounds of the patients who were EIC positive compared with those who were EIC negative with respect to family history of breast cancer, age at first child, etc.?

DR. ALEXANDER J. WALT (Detroit, Michigan): To paraphrase a reknowned slogan, "When the Joint Center for Radiation talks, everyone listens," so the recommendations of this group have a profound impact on that sometimes unrecognized world beyond the boundaries of Boston. Bill Silen and Jay Harris, et al, have always been meticulous in their analyses of their data, and they have long been proponents of conservation therapy for breast cancer. In this they remain well ahead of current practice in the United States. Despite events such as the June 1990 NIH Consensus Conference, one senses an unfortunate national reluctance to abandon or retreat from mastectomy for T1 and T2 lesions. This is evidenced by the fact that in many states, including the State of Michigan, whose figures I have, conservation therapy for T1 and T2 lesions still remains something under 30%.

This is not the time to review all the inhibitory factors that determine this reluctance, but fear of local recurrence is certainly one of them—and I think that is what this paper is all about. Surgical life is a series of trade-offs and this is highlighted in the treatment of stage 1 and stage 2 breast cancer. The conflict here lies between maximum local control and optimal cosmesis.

In the 11% of women in this and other series who have a local recurrence, three things happen: first, the event is very frightening to the patient and disturbing to her surgeon; second, the mastectomy that we hoped to avoid happens, and inevitably the patient is let down psychologically; third, there may be a small but definite negative impact on ultimate survival. At this point, both surgeons and patient are prone to think—if not to say—"would all this have been avoided if just half an inch or 1 cm more tissue all around had been taken, and would this cosmetic difference have been an unendurable or a serious disability?" That is the question. Do you remove a golf-ball-sized or a ping-pong-ball–sized amount of breast tissue?

The Boston group are advocating that the extent of any *in situ* carcinoma present be the determinant of the extent of resection. Let us temporarily accept this argument while recognizing that not everybody, including the NSABP, agrees with it. If one views the invasive carcinoma as the bull's-eye of a target, then each concentric ring beyond it has a diminishing incidence of *in situ* breast cancer. And in case any *in situ* lesion is left in place, we add radiation therapy in the hope that it will sterilize the area. But to rely on radiation therapy to finish the job carries some danger, and may be analogous, in a sense, to the use of antibiotics to clear a residual abscess.

When we look at Bill Silen's T2 EIC-positive group, the recurrence rate was 36% when 3.2 cm^3 was removed. When the excision was stepped up to 4.2 cm^3, the recurrence rate was only 9%. So it seems that the extra centimeter of excision was of great importance and would lend support to Veronesi's data, in which he had a local recurrence rate of about 2% with generous excision. So we are really much influenced by cosmesis.

In this arena, as in so much else in breast cancer today, the patient and the doctor together have to decide about the type and the extent of surgery that one is going to select. Local resection, when carried far enough, ultimately becomes a mastectomy. What has been reported here today helps us to sharpen our decision-making processes.

My questions then are as follows: In the period you described to us, 1968 through 1986 or so, I presume that some modified radical mastectomies were done for T1 and T2 lesions in your institution. Could you tell us what that figure was over that time and what your current figure is?

A second question: You have given figures for local recurrence according to your predefined margins. What was the recurrence rate in the rest of the breast? It is important to include that because recurrence elsewhere in the breast obviously adds to the ultimate mastectomy figure.

A third question is: EIC positivity is central to your decision-making. Do you rely on frozen sections for determinations of the extent of the *in situ* component and, if not, in how many did you have to do a second operation after receiving the fixed section?

And lastly, is there any improvement in outcome among the 125 patients in your series who had adjuvant chemotherapy added to their local resection and irradiation?

DR. WILLIAM WOOD (Atlanta, Georgia): May I have the discussion slide, please? Today's presentation helps to clarify the controversy that has surrounded EIC by suggesting why some institutions have been able to reproduce this finding and others have not. The University of Pennsylvania has long encouraged generous margins in excisions for their breast conservation specimens, and in the BO6 trial, the NSABP did mastectomy for all patients who had marginal invasion or marginal tumor.

Consequently, only in the Institute Curie data do we not really have an explanation as to why they did not see a local failure difference with the presence of EIC. The difference that was demonstrated in Dr. Veronesi's Milan II trial between quadrantectomy (phonetic) and lumpectomy is really fairly complex, because they did not require in their excision or lumpectomy data any clear margins at all, and it includes patients with quite dirty margins, and they also gave less radiation to the patients who had excision than they did to the patients who had quadrantectomy, so that that is a fairly complex study and analysis.

I have two questions for Dr. Silen. In your T2 tumor group, 2 to 5 cm in diameter, if a 3-cm tumor was removed in the smaller group, it would have had a 2-mm or less margin, and the tumor larger than 3 cm would have had no margin at all. You recommend a 1-cm margin at least.

In Dr. Holland's data that you mentioned in the paper, there is extensive tumor going out, usually at least 2 cm and often 3 or 4 cm, yet the addition of only 3 mm more, as was just mentioned by Dr. Walt, was sufficient to take the volume here into the largest group and significantly diminish the incidence of local failure. Would you comment on that disparity?

Secondly, with such narrow to nonexistent margins in the small-volume group, did you calculate the failure rate when you excluded those patients who were known to have a positive margin, and were the data still significant?

DR. ROGER S. FOSTER, JR (Burlington, Vermont): This is an extensive series of patients. They have been well followed, and the study may be shedding some light on the reasons for the controversy over the significance of an extensive intraductal component as a risk factor for recurrence after conservative surgery for invasive ductal carcinoma.

Approximately half of the studies of conservative surgery and radiotherapy have found a correlation between extensive intraductal carcinoma and recurrence, whereas half have not. Dr. Silen and his coauthors have concluded that the influence of microscopic margin involvement on the likelihood of local recurrence has not been established, but that extensive DCIS has an important influence on recurrence.

But in using volume of tissue resected as their measurement, I suspect they are simply using that as a surrogate measure for the histologic margin of resection. I suspect that those groups that base the extent of their resection on careful histologic assessment of margins, including excision of adjacent DCIS, have been doing just what Dr. Silen and his colleagues are recommending. Could it be that it is the groups that take care to obtain histologically clear margins as well as those groups that carry out very wide resections on all patients, that are the ones who do not find a correlation between extensive intraductal carcinoma and recurrence?

The analysis carried out at the Institute Curie found that extensive DCIS, younger age, intralymphatic extension, and adequacy of gross margins all correlated with local recurrence on univariate analysis, but when they carried out a multivariate analysis, extensive DCIS was no longer significant.

My question is: Have you carried out a multivariate analysis, particularly with respect to age, that might alter the conclusions?

DR. WILLIAM SILEN (Closing discussion): Dr. Zeppa, in answer to your question about the background family history, age at first child, and so forth, there was no difference in these various groups.

Dr. Walt, the incidence of mastectomy has changed, and it continues to change in our series. Early in the series, certainly 70 or 80% of the patients were undergoing mastectomy, and then as conservative treatment became more comfortable, more patients underwent it, local excision, and radiotherapy; and now I think we are swinging back a bit more, so that I would say that roughly 30% or 40% of the patients are having mastectomy at present.

This correlates reasonably well with the overall incidence of extensive intraductal carcinoma, which in our overall series runs somewhere around 30%. And it is those patients who tend to have mastectomy.

The incidence elsewhere in the breast that you asked about was 1%, and so we put those out.

In terms of how often do we re-resect, I would say that it is very often in our current protocol, because of the fact that so many of the patients come to operation because they have no palpable mass but have a needle localization and the microcalcifications are removed, and then we need further tissue. So I would say that at least 30 to 40% of the patients undergo reresection.

The concern that I had about all this was that the survival data may be influenced if we are going to have recurrences in the breast. To date the analysis suggests that it has not influenced survival, and I think there are other data in the literature that suggest that local recurrence, when followed by a mastectomy, does not influence survival adversely.

Dr. Wood, thank you very much for your comments. In terms of the presence or absence of the EIC, I think this is very difficult in explaining the disparity in size that we are talking about in terms of volume, because if one looks at these, they are little, tiny foci, one little tubule or ductule of noninvasive carcinoma that sits out there somewhere in the periphery, and this makes it very difficult.

We are currently trying to evaluate margins, and I can assure you that thousands of slides are being made by our pathologists and it is, I think, extremely difficult, if not impossible, to evaluate margins.

Dr. Foster, we did evaluate age in a multivariate fashion, and indeed the incidence of extensive noninvasive carcinoma is much higher in the young person. When we took that into account, the outcome was still the same.

NSABP Trials of Adjuvant Chemotherapy for Breast Cancer

A Further Look at the Evidence

C. BARBER MUELLER, M.D.,* and MARY L. LESPERANCE, PH.D.†

From the Departments of Surgery and Mathematics and Statistics,† McMaster University, Hamilton, Ontario, Canada*

Two decades ago the National Surgical Adjuvant Breast Project (NSABP) began to test the hypothesis that adjuvant chemotherapy would influence favorably the postsurgical survival rates of women with breast cancer. Although the use of phenylalanine mustard (melphalan or (L-PAM) has been supplemented by other agents, the thrust to recommend adjuvant chemotherapy as standard treatment was created by an L-PAM series of five trials using L-PAM or L-PAM plus fluorouracil. Only the first trial, B-05, contained an untreated group. The main treated groups in these five trials are similar to each other in survival probabilities, and the main untreated group in B-05 is not different in survival than the combined results of these five trials. There were a total of nine comparisons in each study—and in B-05 the subgroup of treated women 49 years or younger with one to three positive node was found to be different than the untreated group. Four subsequent subgroups of women who were 49 years or younger with one to three positive nodes are not similar in survival despite the fact that they were developed from similar main groups that had similar treatment. The initial treated subgroup in B-05 had a survival outcome that was never again achieved in the subsequent four studies. The untreated subgroup in B-05 was not different than three of the four subsequently treated subgroups. The NSABP now has evidence that denies the conclusion that adjuvant chemotherapy prolongs survival in premenopausal node-positive women. Recommendations that adjuvant chemotherapy become standard treatment for premenopausal node-positive women should be seriously reconsidered and probably withdrawn.

ADJUVANT POSTOPERATIVE chemotherapy for premenopausal node-positive breast cancer patients became therapeutic policy 15 years ago because, despite its accompanying illness, it seemed to hold promise for surviving a disease that is so devastating. The National Cancer Institute (NCI) and the National Surgical Adjuvant Breast Project (NSABP) have been the major United States advocates. In several publications Fisher[1-6] has assumed that the findings of the first major NSABP study, B-05, firmly established that adjuvant chemotherapy improves the survival of premenopausal node-positive women. There has never, however, been a subsequent study to ensure that the untreated women in this study represent the world at large or that the treated women have a survival record that can be duplicated. In fact subsequent NSABP data fail to do either. Using NSABP data this review not only questions the basic NSABP assumption but also challenges the conclusion that the administration of adjuvant chemotherapy gives survival benefit to premenopausal node-positive women.

The Basic Data

NSABP has conducted five studies of node-positive women treated with combined phenylalanine mustard (melphalan or (L-PAM) (P), B-05, B-07, or L-PAM plus 5-fluorouracil (PF), B-08, B-09, B-10 (Fig. 1).

Only B-05, the initial study, contained an untreated (placebo) group, with 170 untreated and 179 treated subjects. The main groups were stratified according to age (pre- and postmenopausal status) and to the number of positive nodes (one to three and four or more). As 1 of 9 comparisons, the subgroup of premenopausal (49 years or younger) women with one to three positive nodes was the only subgroup to show a statistically significant difference in survival rate. It contained 32 treated and 31 untreated women and at 12 years there were 10 more women dead in the untreated than in the treated group. If four women had died differently, the result would not have been statistically significant.[7] B-05 was followed by four other studies in which all subjects were treated. Data from the subsequent four studies now afford an oppor-

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Address reprint requests to C. Barber Mueller, Department of Surgery, McMaster University Medical Centre, 1200 Main St. West, Hamilton, Ontario, Canada, L8N 3J9.

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THE L-PAM SERIES

5 STUDIES - SURVIVAL

B - 05	P	- PLACEBO
B - 07	P	- PF
B - 08	PF	- PFM
B - 09	PF	- PFT
B - 10	PF	- PFcP

P = L-PAM
F = FLUOROURACIL

FIG. 1. A list of the five L-PAM trials. The five control arms enclosed in the dotted box constitute the data used in this review, against which the untreated (placebo) arm is compared. M, methotrexate; T, tamoxifen; cP, *C. parvum.*

tunity to look at main group similarities, or differences, between the five studies (five treated arms and one untreated) and to look further at the effects of treatment on premenopausal women (those 49 years or younger) with one to three positive nodes.

B-05 and the L-PAM Series—The Main Groups

In 1972 the first of five studies using L-PAM as adjuvant therapy to node-positive women was initiated. This is the most significant of the five studies because it contains the only untreated group and everything subsequent to this study depends on the correctness of differences between its treated and untreated groups or subgroups. The subsequent four studies were similarly stratified for pre- and postmenopausal women and for one to three and four or more nodes (Fig. 2).

The main groups in B-05 and B-07 were treated with L-PAM, and in B-08, B-09, and B-10 with L-PAM plus fluorouracil. Treated groups in these five studies constitute nine independent studies but this review uses only the five control arms (boxed in Figure 1) because no significant difference between control and experimental arms was demonstrated in the last four studies (Table 1).

The main treated groups constitute five independent studies that may be compared with one another as well as with the one untreated group of B-05. Tests for between-study equivalence (log-rank statistic p = 0.133) indicate that the five main control arms are not different from each other. The estimated survival curve of the untreated group is significantly different from the estimated survival curves of the control groups of B-08 and B-10, but it is especially noteworthy that the survival curve of the largest group (B-09) with 924 subjects at the study onset is not significantly different from the B-05 untreated group of 170 subjects (log-rank statistic p value = 0.114.[8]) Figure 3 shows the estimated survival curves and confidence intervals for these five consolidated main groups compared to the untreated group from B-05. Confidence intervals, narrower for the consolidated group because of the large number of subjects and wider for the untreated group because of the few subjects, overlap considerably, indicating no significant difference in survival outcome between the many controls and the untreated.

It is thus seen that all five of the NSABP main study groups, similar at entrance, with similar treatments, have similar survival data. When these five groups are compared to the one untreated group from B-05, there is no convincing evidence that adjuvant chemotherapy has made a difference in survival.

The Premenopausal (49 Years or Younger), One to Three Positive Node Subgroups

Each of the five studies also tabulated results for subgroups of premenopausal women with one to three positive lymph nodes. In B-05 a statistically significant difference in survival was found between the treated subgroup of 32 women when compared to the 31 untreated (Table 2). Despite the small sample size, the scientific, statistical, and clinical reliability of this finding was never again tested because it was considered unethical to ever again have an untreated cohort. There seemed to be assurance that these 31 women adequately represented the United States population of premenopausal women with one to three positive nodes and that the 32 treated women had survival results that could be duplicated in subsequent efforts. There has been no subsequent attempt

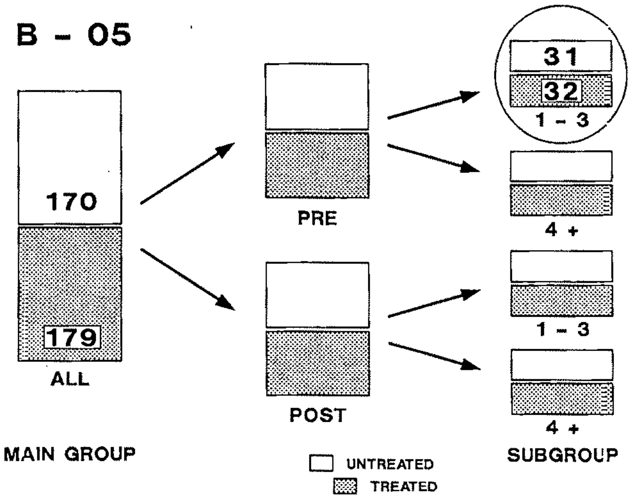

FIG. 2. This is the general outline of all five trials showing the main randomization with stratification for premenopausal (≤49 years) and postmenopausal (≥50 years) status followed by extent of nodal involvement (one to three and four or more positive axillary nodes). This figure shows the number of subjects at risk in the two main arms of the B-05 trial and the number at risk in the patients who were 49 years or younger with one to three positive nodes.

TABLE 1. *Main Groups (% Survival ± 0.95 Confidence Interval)*

	Years	2	5	7	10	12
Placebo	(170)	85 ± 5.4 (145)	63 ± 7.3 (107)	54 ± 7.5 (91)	41 ± 7.4 (70)	38 ± 7.3 (63)
B-05 P	(179)	88 ± 4.8 (158)	66 ± 6.9 (119)	58 ± 7.2 (104)	47 ± 7.3 (84)	43 ± 7.3 (74)
B-07 P	(345)	89 ± 3.3 (308)	63 ± 5.1 (217)	56 ± 5.2 (189)	47 ± 5.3 (154)	42 ± 5.3 (117)
B-08 PF	(356)	93 ± 2.7 (330)	70 ± 4.8 (250)	61 ± 5.1 (216)	51 ± 5.2 (176)	
B-09 PF	(924)	91 ± 1.8 (835)	67 ± 3.0 (616)	55 ± 3.2 (501)	44 ± 3.4 (139)	
B-10 PF	(125)	98 ± 2.5 (122)	75 ± 7.6 (94)	(8 yrs) 57 ± 8.8 (61)		

Survival data and numbers at risk for the placebo arm of B-05 and the control arms for all five L-PAM trials.

P, L-PAM; F, fluorouracil.
( ), number at risk.

to test the former or substantiate the latter. A consensus conference called by NCI on the basis of this trial[9] recommended that adjuvant chemotherapy become standard therapy. The four subsequent L-PAM studies now provide an opportunity to compare the B-05–treated subgroup to the other four treated subgroups that come from trials similar in entrance criteria, treatment, and survival to each other and to compare the untreated 31 subjects to each of these. These five treated subgroups are not similar among themselves—log-rank statistic p = 0.075. The 10-year survival probability of 81% found in B-05 was never again achieved. B-07 and the large B-09 study each had 10-year survival probabilities of 62%. B-10 had a 62% survival probability at 8 years. Estimates of survival probabilities and the confidence intervals for each of these treated subgroups have been compared to the small (31 subjects) untreated subgroup of B-05 separately. Three of the five treated subgroups do not differ significantly from the untreated 31 of B-05. It is particularly noteworthy that the large B-09 PF study with 211 subjects is found to be not significantly different from the untreated 31 subjects of the B-05 study (Fig. 4).

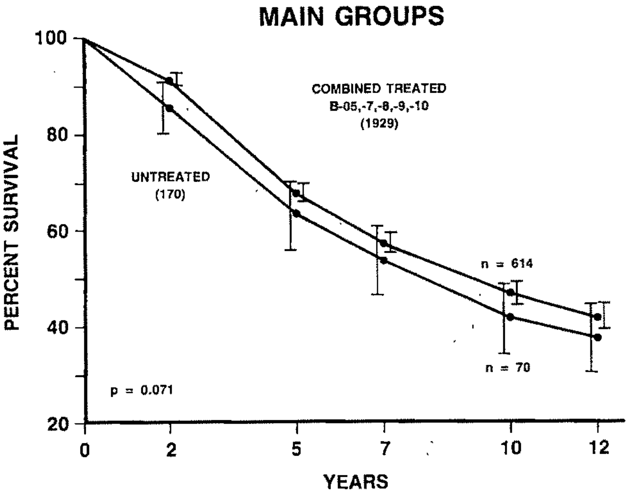

FIG. 3. Survival curve of 170 untreated subjects compared to the survival curve of the combined 1929 treated subjects in the control arms of all five L-PAM studies. Confidence intervals (0.95) show overlap, which indicates no statistically significant difference.

It may be concluded that the subsequent four studies—B-07, B-08, B-09, and B-10—do not support the conclusion that subgroups of treated women 49 years or younger with one to three positive nodes survive longer than a comparable untreated subgroup.

Discussion

The main groups in these five studies are similar in survival, there are little differences in therapy, and initial selection and stratification successfully produced five similar main study groups. The subgroups—women of 49 years or younger with one to three positive nodes—which come from main groups similar in selection, treatment, and survival are not similar to each other in survival. In these early years, stratification by age and nodal status did not take into account other features that have been shown subsequently to be important and to influence survival. Fisher,[10,11] in reviewing the pathologic specimens of all patients in B-04 group (the radical *versus* simple mastectomy trial), listed 24 pathologic features, among which nuclear grade and histologic grade were found to be associated with short-term treatment failure. It is not known, for example, that nuclear grade was evenly distributed in the two subgroups of B-05, the 31 *versus* 32 women. Subsequently ER, PR, ploidy, S-phase fraction, and other features of the primary tumor have been found to influence survival rates.

In large groups it is hoped that random allocation distributes these many unstratified elements fairly evenly. If small subgroups derived from similar main groups are

TABLE 2. *NSABP Data in Subgroups of Patients 49 years or Younger with One to Three Positive Nodes*

	Years	2	5	7	10	12
Placebo		94 ± 8.4	74 ± 15.4	65 ± 16.8	48 ± 17.6	45 ± 17.5
	(31)	(29)	(23)	(20)	(15)	(13)
B-05 P		97 ± 5.9	88 ± 11.3	81 ± 13.6	81 ± 13.6	78 ± 14.4
	(32)	(31)	(28)	(26)	(25)	(23)
B-07 P		95 ± 4.9	79 ± 9.2	76 ± 9.7	62 ± 11.2	59 ± 11.4
	(76)	(72)	(58)	(55)	(44)	(38)
B-08 PF		100	82 ± 8.4	80 ± 8.7	77 ± 9.3	
	(80)	(80)	(66)	(63)	(56)	
B-09 PF		96 ± 2:7	78 ± 5.6	68 ± 6.3	62 ± 7.0	
	(211)	(201)	(163)	(141)	(40)	
				(8 yrs)		
B-10 PF		97 ± 5.5	78 ± 13.3	62 ± 16.9		
	(37)	(36)	(28)	(20)		

Survival data and numbers at risk for the untreated subgroup (patients who were 49 years or younger) with one to three positive nodes from B-05 and the five treated subgroups of patients who were 49 years or younger with 1 to 3 positive nedes.

P, L-POAM; F, flucrouracil.

( ), number at risk.

not similar, it is possible that these unnoted important elements are not equally distributed and it is probable that it is their influence, rather than treatment, that caused the statistically significant difference between the subgroups of women who were 49 years or younger with one to three positive nodes who were treated and untreated in B-05. This would explain why the five comparable L-PAM–treated subgroups are statistically different from each other and why the survival outcomes of some of them are similar to that of the untreated subgroup of B-05 (Fig. 5).

Groups or subgroups under study must have some relationship to the population at large before conclusions drawn from the study can be recommended as general principles for care. This is a critical sampling problem and to have made national policy on a sample of 31 untreated women seems to have been premature. Subgroup analysis of a prospective trial is legitimate and interesting but any conclusions drawn from it require rigorous scrutiny in subsequent trials. It is inappropriate and unethical to make far-reaching practice recommendations on subgroup analyses because the number of subjects is small, the equivalence of subgroups is open to question, and confidence limits are wide. The subsequent L-PAM trials do not support the early NCI recommendations for standard care that were made on the basis of the B-05 experience.

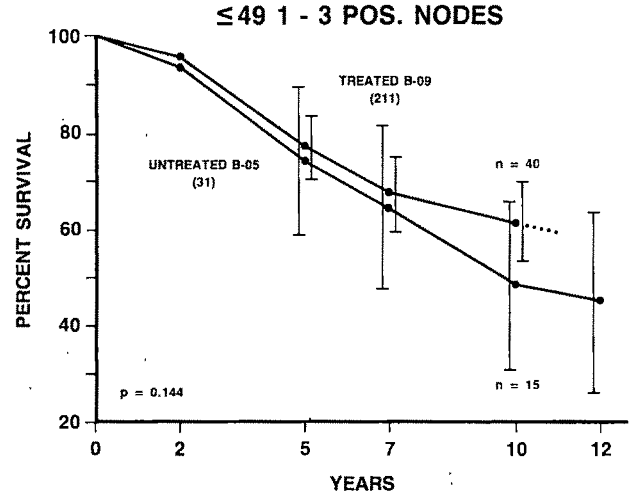

FIG. 4. Survival curve of 31 untreated subjects who comprise the B-05 subgroup of patients who were 49 years or younger with one to three positive nodes compared to the comparable large subgroup of 211 PF treated subjects collected in B-09. Confidence intervals (0.95) are shown for both subgroups, indicating no statistically significant difference.

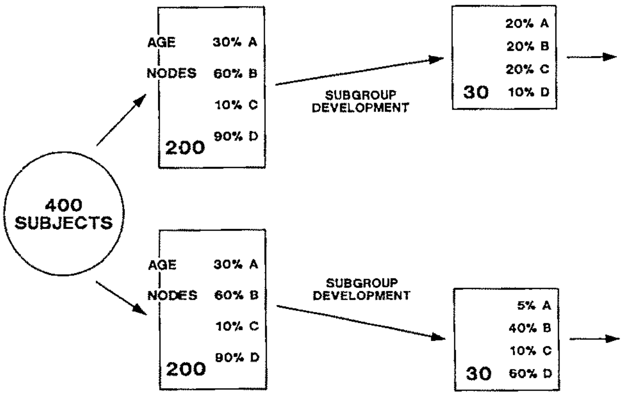

FIG. 5. A model to describe the process whereby randomization and stratification produce two main groups equal for age and nodal status also equally stratified for important unknown and unrecorded elements. Subsequent subgroup development, however, does not guarantee that these unrecorded elements are equally distributed in the subgroups. If they are important to survival, they may be the determinant of any difference that is found, rather than treatment given to one subgroup compared to another.

A critical review of 39 randomized trials of adjuvant therapy for breast cancer by Wilson, Houghton, and Baum[12] found that only three convincingly demonstrated survival advantages. A recent report on the Guys/Manchester trial of cyclophosphamide, methotrexate, fluorouracil (CMF) as adjuvant therapy shows a small survival benefit in the premenopausal women who were treated. This benefit appears to be associated with the development of amenorrhea and again the unsettled question arises as to whether the benefit is due to endocrine deprivation rather than a cytotoxic effect *per se.*[13]

A meta analysis from England[14] concluded that at 4 years there may be a 7% survival advantage of 10 months when multiagent adjuvant chemotherapy is given to premenopausal women with positive nodes. This meta analysis requires the assumption that breast cancer is the same all over the world and that world-wide studies can be compared with each other. Despite the knowledge that attack rates are dissimilar, no one has attempted to confirm that untreated breast cancer in Milan or London is the same as it is in the United States.

Conclusions drawn from the report on the B-05 subgroup of women who were 49 years or younger with one to three positive nodes have been far reaching, with a major impact on physicians, oncologists, trial organizers, ethicists, and patients. The result are not convincing that the P and PF arms of the B-05 through B-10 trials yield marginally higher but not statistically significant survival probabilities than the untreated arm of B-05. Observations on the women selected in B-05 require further validation to be certain that conclusions are based on real treatment differences and not on differences due to unknown bias between the untreated arm of the B-05 subgroup and all other treated comparable subgroups.

NSABP evidence on the results of treatment of premenopausal node-positive women in B-05 led to a consensus conference that recommended that such women be treated with adjuvant chemotherapy. Subsequent data generated by NSABP do not support the conclusions of B-05 and undermine the recommendations of the consensus conference. The treated subgroup (women who were 49 years or younger with one to three positive nodes) of 32 women in B-05 with 81% 10-year survival is unlike any other treated subgroup. The untreated subgroup (women who were 49 years or younger with one to three positive nodes) of 31 women has never been examined to see if it accurately represents the entire population of premenopausal women with one to three positive nodes.

Summary

A. The five L-PAM trials
   1. The main treated groups of the five trials in the L-PAM series are similar in survival probabilities.
   2. The main untreated group in B-05 is not different than the combined results of the five trials.

B. The treated subgroups (women 49 years or younger with one to three positive nodes)
   1. Come from similar main groups
   2. Are similar in treatment received
   3. Are not similar in survival outcome
   4. Therefore are probably not similar in initial stratification

C. The two B-05 subgroups (women 49 years or younger with one to three positive nodes)
   1. The treated group had a survival outcome not achieved in three of the four subsequent studies.
   2. The untreated group had a survival outcome similar to that of three of the four subsequent studies.
   3. It has not been ascertained that the untreated subgroup of 31 women was representative of the world at large.

Conclusions

The NSABP has now produced evidence that adjuvant chemotherapy is not significantly effective in premenopausal node-positive women. Thirty-one untreated women is too small a sample of premenopausal United States women with breast carcinoma to be considered representative of the entire population, particularly when a difference of only four deaths would have eliminated the statistical significance found in the initial study—B-05. The NCI/NSABP recommendations based on previous work (B-05) are outdated and need review. And finally the recommendation that adjuvant chemotherapy become standard treatment for premenopausal node-positive women should be seriously reconsidered and probably withdrawn.

Acknowledgments

The authors thank Professor Robin Roberts, Department of Epidemiology and Biostatistics, and Dr. Charles Dunnett, Department of Mathematics and Statistics, for their contributions made to this analysis.

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DISCUSSION

DR. W. P. MCDERMOTT (Boston, Massachusetts): These two papers emphasize not only the tremendous importance of carcinoma of the breast in the female population of this country and the steadily increasing interest that has appeared over the years in attempting to evaluate and treat the disease, but Dr. Mueller in particular has presented a nihilistic view, one aspect of the problem; I would like to stand and take a positive view, but I am afraid I cannot.

I would like to report to you a small, very small, subgroup that was of interest to us, to see if we could cast some light on some of the peculiarities that we recognize as being involved in the treatment and the prognosis of cancer of the breast. To evaluate some of the factors involved and the degree of malignant potential of this cancer, all the late recurrences of cancer of the breast, at more than 10-year intervals seen over a 20-year period, have been reviewed.

To achieve this we had to go well back in history. For a period from January 1965 through December 1984, 3855 invasive cancers of the breast were seen either at the initial diagnosis or later in the disease. In this group there are 29 cases in whom the first evidence of recurrent disease appeared 10 or more years after the initial diagnosis was established, and this was the group that intrigued us and that we tried to evaluate.

The interval between recognition and recurrence ranged from 10 to 24 years, a median of 14.5, a mean of 13.9. Age of patients at onset was 30 to 71; median, 48; mean, 50.4. Nine patients were younger than age 45; only one was over 70. Obviously to get a late recurrence one has to live long enough. Twelve measures of receptors were carried out; all except one were elevated. Nineteen patients had nodes examined; of these, 10 had one or more axillary nodes positive (two had more than 10). Only four of the tumors were classified as undifferentiated. In eight of the patients, the initial recurrence was local; in 21, distant. In the eight patients with local recurrence, six shortly thereafter developed distant metastases. Two remained free of disease after excision.

With varying types of therapy, nine patients are still living, one to 20 years (with a median of 5 years after recurrence), but only two have no evidence of disease. Of the 20 deaths (all except one of disease), the survival data ranged from 52% at 5 years to 18% at 10 years after the initial recurrence.

You can see from this, that, at the beginning of the 10-year point when the first recurrence was apparent, the curve of survival after this really is very similar to the overall pattern that one sees in broader or extensive studies of cancer of the breast from the initial therapy, which just introduces another peculiar, nihilistic view of our knowledge of the behavior of this tumor.

These data on an unusual subset of patients would support the concept that the course of most cancers of the breast is dependent primarily on the malignant potential of the cell and the relationship to an individual's immune system rather than on any type of local, regional, hormonal, or chemotherapeutic manipulation.

A subsequent series in which studies are available, such as ploidy flow cytometry, angiogenesis, etc., may provide more predictive information than is available in this series, in which the most recent case (because of the long interval) in which a diagnosis was first established occurred more than 15 years ago.

Well, this is nothing except further nihilism, but it was at least of interest to us and I hope, to some extent, to you.

DR. WILLIAM WOOD (Atlanta, Georgia): The discussion of Dr. Mueller is a very delightful and careful dissection of these data from the NSABP. One of the concerns when these data appeared and afterwards was that not only were the numbers small, but the possibility exists of a very real publication bias. When a study is positive, one is eager to publish it; when a study is negative, one is perhaps willing to wait longer to see if it will become positive before publishing it. If one decides to publish it, one often has difficulty finding a journal that is eager to reproduce it.

And consequently, the Early Breast Cancer Trialists came together as a group of investigators who wished to track down all randomized adjuvant breast trials anywhere in the world and analyze their data to see if we were seeing a publication bias that was rather triumphalist.

When eight randomized trials were compared from around the world, including the BO5 trial that you have just reviewed, the investigators looked at single-agent chemotherapy, like BO5, in over 1000 women less than 50 years of age, and the conclusion of the analysis of all these trials, just as Dr. Mueller has concluded about this trial, BO5, is that there was no significant benefit at all in overall survival.

And consequently in the National Institute of Health (NIH) consensus recommendations of a few years ago, these data were not considered, but in 27 randomized trials involving 2526 SACH women who were randomized to receive multi-agent chemotherapy, usually CMF (cyclophosphamide, methotrexate, and fluorouracil), there was a 26% lower rate of death—not of disease-free survival, but a lower rate of death—over more than 5 years, using combination chemotherapy, as published in the *New England Journal Medicine* just over 2 years ago, with a p value of less than 0.001.

Now, with over 10 years of follow-up of over 3300 women less than 50 years of age so randomized, the survival benefit has actually increased over the second 5 years.

Are you, Dr. Mueller, advocating multiple-agent chemotherapy instead of single-agent chemotherapy, or are you challenging the value of adjuvant chemotherapy in its totality for these women?

DR. FRANK E. GUMP (East Orange, New Jersey): There is no question that adjuvant chemotherapy continues to be one of the more contentious areas of breast cancer treatment. I think that the debate, at least in the last few years, has primarily focused on social and economic factors, and statements vary all the way from that it is the Halsted radical of the 1980s—that is Rose Kushner—to that it is an advance of monumental proportions. That is James Holland in the *New England Journal.*

It is in this setting that Dr. Mueller's contribution is so noteworthy, because he has forced us to go back and look at the underlying hypothesis. Does adjuvant chemotherapy reduce mortality from breast cancer?

I cannot find any flaw with the way he has analyzed these data, and more to the point, I think it is perfectly proper to group treated patients in these trials and to compare them with the placebo group, and analyzed in that way, I do not think we can fault the conclusion that the hypothesis does not pass. So in my book, Dr. Mueller has certainly won the battle. The more difficult question is, has he won the war?

We have to start off by saying—and this has been mentioned by Dr. Wood just a minute ago—that the medical oncologists have long since discounted the LPAM trials, believing that this is not a particularly potent agent, and multiple agents are needed to get the best results.

Certainly these drugs do something. It is not like giving peppermint water; to use Michael Baum's words, the natural history of breast cancer has been perturbed, and I think that has got to be the perfect word for all of this. I still have to come back to the basic question, which in my mind is, can you ever kill 100% of all these cells, no matter how minimal the tumor burden? Or to put in another way, I would certainly feel very differently about chemotherapy if I could find one patient with known disease where that disease was eradicated by chemotherapy.

Now admittedly this is possibly a very unrealistic or unreasonable goal, but when you look at these trials, I do not think you can ever fail to be impressed by the marked disparity between disease-free survival and survival. And although this is usually attributed simply to the slow progression of breast cancer, I wonder, Dr. Mueller, if you might comment on another possibility: when disease-free survival is the end point, you're comparing chemotherapy with no treatment. When survival is the end point, however, you're comparing chemotherapy given postoperatively with chemotherapy given at the time of first recurrence.

And it could be that this mysterious and certainly unmeasurable entity of tumor burden is simply not that different in these two periods, especially in the context of a trial when people are being followed very closely for the first evidence of recurrence.

Finally I certainly believe that any worthwhile organization needs a contrarian, and we are very fortunate to have Dr. Mueller among us.

DR. WILLIAM S. BLAKEMORE (Birmingham, Alabama): This is another of many contributions for which Dr. Mueller should receive credit. None of us can endorse the excision of a few more millimeters of tissue nor the results of a clinical trial in which the shift of a few patients makes a great difference in the result. Still I know of no other mechanism available to study some of these diseases that require such long-term follow-up than by clinical trials.

When Dr. I. S. Ravdin was recruited to organize adjuvant studies in 1956, he did so reluctantly. One of the factors that persuaded him was that the surgical community would establish leadership in continuing to take complete and extended care of these patients. Considerable credit must be given to those who have continued the trials, namely Dr. George Moore and certainly Dr. Bernard Fisher, who have tried, to the extent that the studies were acceptable to the clinicians and within ethical standards, to do the studies and establish statistically valid scientific results.

A number of other preliminary studies have been lost along the way because all could not be followed by extensive trials. One of those was by Dr. Thomas Nealon, who more than 10 years ago used unfavorable histologic characteristics to select a group of patients with early breast cancer who would have favorable results. We have taken some of the recent studies with ploidy and used his characteristics to show that there are better predictive values with the combination of his studies and ploidy studies.

He defined the low-risk, intermediate-risk, and high-risk groups by the size of the tumor, blood vessel and or lymphatic invasion, marginal infiltration, and nuclear grading. We have been able using ploidy studies to determine a group of patients with T1/T2NoMo lesions who have low-risk with 98% 10-year survival.

The intermediate group were those in which he had one or more of his unfavorable characteristics or T2 tumor, which again can be separated by those with euploid characteristic having 100% 10-year survival, and those with aneuploid characteristics having 67% 10-year survival. A high-risk group with euploid and aneuploid findings had a 10-year survival of 95% *versus* 45%. In the latter group, some type of treatment must of offered. Most of us agree that 45% survival still is a challenge to our management.

Patients who had local recurrence with euploid tumors, with the exception of one patient, survived with local excision. Those patients with aneuploid tumors with recurrences all died.

As these factors are being presented, there is a litany of things that promise to be of use clinically. These define genetic, growth, and metabolic factors: euploidy, aneuploidy, and tetraploidy, etc. These may require computerization, now called networking, to weigh the importance of various factors alone or in combination.

I would like to appeal for leadership from the surgeons to provide the continuity of care in breast cancer. I hope that some of you will develop a better system than the NSABP trials, but I do not know of any available.

DR. C. BARBER MUELLER (Closing discussion): Dr. McDermott, I was interested to hear about your small subgroup. It took me back to this meeting in Quebec City, at which time I presented the consequences of a long look at what happens to women who get breast cancer. Looking at the cause of death at 15 years, almost all were dying of breast cancer. This information was received with a great deal of skepticism, but subsequent studies from Cambridge and elsewhere that took a look at those women who died 10, 20, and 30 years after mastectomy found that many of them, if not most of them, continue to die of their breast cancer. It is a bad disease.

Dr. Wood, as to publication bias—I am aware of the early Trialists' Collaborative report. I think this is headed by Peto Dos Metalanisis. Dr. Lesperance is a statistician and a mathematician, not a surgeon, so I asked her to look at that report. I asked what would happen if B-05 were withdrawn from that. She said that there would not be any statistical significance because this was one of the big controls. She said if you took the entire meta-analysis, it probably would not make any difference.

But when you look at the figures from the meta-analysis, Dr. Wood said that there is a 28% improvement in survival. This is a relative figure, not an actual. The actual figure is approximately 7% of the women. If one looks at the entire study, there may be a 10-month delay in death in 7% of the group by the administration of adjuvant therapy. If one looks at the absolute, the actual result, rather than the relative, the numbers look very different. Also you must look at the X-axis as well as the Y-axis.

And to continue, Dr. Woods, you asked if I would advocate multi-agent adjuvant therapy,—or none—to a premenopausal stage II woman. Evidence for multi-agent therapy against no therapy has yet to be developed with sufficient strength to make a recommendation that would be in her best interest. If she wishes to be a subject in an experiment, I would encourage her—but it should be one that asks a critical question, not one that attempts to confirm a preconceived position.

Dr. Gump, thank you for your kind comments. I think that I, too, know that the LPAM material has been discarded and that more powerful drugs are being used, and I guess my concern is that the hoopla that followed the initial study has not subsided or has not been discarded.

Michael Baum, in an adequate review of 36 trials of adjuvant chemotherapy in breast cancer, found that only three contained convincing evidence of benefit. When one looks at the meta-analysis, the odds ratio does not move very far from that up-and-down line that denotes equality between treated and untreated results.

You also talked about disease-free survival *versus* survival. I thank you for bringing that up. I do not think I planted it with you, but a month ago at the Central Surgical, I attempted to deal with some of the articles—and there are quite a few—that reported prolonged disease-free interval but no difference in survival. I concluded that this meant there was a shorter time from recurrence to death in those who had received adjuvant chemotherapy than in those who had not, and attempted at that time to pose five possibilities as to why this may occur.

You may read about the five in the journal *Surgery*. It will be out in October or November. I concluded that antineoplastic drug resistance was induced by the adjuvant chemotherapy, and that the treatments called salvage treatments or postrecurrence treatments were less effective in the group who had received adjuvant chemotherapy than in those who had not. This is not unlike the experience with oophorectomy, and we are right back again to the business of "do you treat everyone" or do you wait to treat those who get the recurrence when they need it?

Dr. Blakemore, thank you for your kind comments. The scientific look, I'm sure will subsequently require a resolution of some of these many factors that are present, but this will take complicated trials. They must be scientific, carefully designed, and, unfortunately, in a disease that is as complicated as this and as prolonged as this, it will not be easy.

Dissociation of Blood Volume and Flow in Regulation of Salt and Water Balance in Burn Patients

WILLIAM G. CIOFFI, JR., M.D., GEORGE M. VAUGHAN, M.D., JAMES D. HEIRONIMUS, M.D., BRYAN S. JORDAN, R.N., M.S.N., ARTHUR D. MASON, JR., M.D., and BASIL A. PRUITT, JR., M.D.

From the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas

The relationship between effective blood volume and related hormones in burn patients following resuscitation is not well understood. Previous reports have suggested that hormone secretion is altered by a resetting of neural control mechanisms. Serum and urine sodium, plasma renin activity, serum ADH, cardiac index, effective renal plasma flow, and total blood volume were measured in seven burn patients (mean age, total burn size, and postburn day: 32 years, 56%, and 9 days, respectively). The same values (with the exception of cardiac index and blood volume) were measured in 10 control patients (mean age, 24 years). The blood volume of patients was measured by ^{51}chromium red blood cell (RBC) labeling and compared to normal predicted values based on body surface area and sex. Mean serum sodium and osmolality were 138 mmol/L (millimolar) and 286 mosm/kg, respectively, in both patients and control subjects. Mean ± standard error of the mean total blood volume in the patients was low, 81% ± 4% of predicted values. Cardiac index and renal plasma flow were significantly elevated. Plasma renin activity and antidiuretic hormone (ADH) levels were elevated and altered in the direction expected from blood volume measurements despite the findings of increased blood flow. Dissociation of organ flow and hormonal response suggests that simultaneous direct blood volume measurements are necessary to elucidate factors other than altered neural control settings to explain hormonal changes in the flow phase of injury. Depressed total blood volume appears to promote elevated ADH levels in burn patients following resuscitation. Whether there is an additional role of altered neural control settings remains to be established.

FACTORS RESPONSIBLE FOR sodium and blood volume regulation following injury are not clearly understood. Several authors have interpreted their data to imply that resetting of hormonal control mechanisms occurs following thermal injury and that this is a stress response that is not sodium or volume dependent.[1,2] Although various studies have examined one or two factors responsible for sodium and volume regulation following thermal injury, no one has studied this system as a whole.

Antidiuretic hormone (ADH) response following thermal injury has been examined recently.[2–4] Morgan et al.[2] have concluded that ADH levels were elevated after burn and remained so for 7 to 10 days. In addition the increased ADH levels appeared to have little relation to serum osmolality and did not affect urine output. Shirani et al.[3] observed elevated plasma ADH levels in association with hyponatremia in burn patients even beyond the first 10 days. Those results were interpreted as being consistent with the diagnosis of the Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH). However in these studies blood or plasma volumes were not measured simultaneously with the measurement of ADH.

The renin-angiotensin-aldosterone axis has been examined following thermal injury.[1] Shirani et al.[3] suggested that the elevated plasma levels of renin activity, angiotensin I, angiotensin II, and aldosterone following thermal injury reflected a resetting of hormonal control and did not depend on an effective plasma volume deficit. No volume measurements were made in that study. In that group of patients, combinations of these hormones remained volume responsive, as verified by saline-loading tests.

Atrial natriuretic factor (ANF), a family of potent natriuretic and diuretic peptides, are present in mammalian cardiac atria. Central hypervolemia and increased blood

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Address correspondence to Maj. William G. Cioffi, M.D., U.S. Army Institute of Surgical Research; SGRD-USC-B; Fort Sam Houston, TX 78234-5012.

Address reprint requests to Library Branch, U.S. Army Institute of Surgical Research, Bldg. 2653, Fort Sam Houston, TX 78234-5012.

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pressure have been postulated as factors that promote ANF secretion.[5] An elevation of ANF has been shown to blunt aldosterone response to stimulation by angiotensin II.[6] The effect of thermal injury on plasma ANF levels and how it, in turn, affects salt and water balance has not been described.

To define more precisely the mechanisms that regulate salt and water balance following thermal injury, we assessed plasma levels of ADH, ANF, and the renin-angiotensin-aldosterone axis simultaneously with measurement of blood volume and osmolality in burn patients 5 to 16 days after burn.

Materials and Methods

Ten healthy control subjects (seven men, three women) and seven burn patients (six men, one woman) whose characteristics are detailed in Table 1 were each studied during a 5-hour period, with blood and urine samples collected hourly for determination of electrolytes and creatinine. Controls were allowed to eat nothing by mouth beginning at 00:00 hours on the day of the study while the patients' enteral feedings were continued but oral intake was held. The patients' intravenous fluids were administered at a rate to maintain adequate urine output while allowing for an approximate 10% daily loss of the weight gain from initial resuscitation. The rate of fluid administration in the controls was matched to the mean patient hourly intake. Five patients weighed more than the preburn weight, while two weighed less than the preburn weight on the day of study (Table 1). The mean values during the study period were used as data for each patient. After a tracer bolus injection of ^{131}I-hippuran (I-HIP) at the second hour (09:00 hours), additional blood samples were taken to characterize I-HIP decay in the plasma. Just before injection of the I-HIP, a plasma or serum sample was taken for determination of hormone concentrations. Cardiac index (CI) was determined by thermodilution at intervals during the study and blood volumes were determined with ^{51}Cr-tagged autologous red cells at the end of the study period. Neither CI nor blood volumes were measured in control subjects.

Sodium and potassium were determined by flame photometry and osmolality by freezing-point depression. Urinary excretion rates and clearances were normalized to 1.73 m^2 body surface area (BSA): square root of [(height in cm × weight in kg)/3600].[7,8] Preburn weight was used to express variables requiring body weight.

TABLE 1. *Demographic Variables*

Variable	Control	Patients
Age (years)	23.8 ± 1.4*	32.4 ± 5.8 (18–64)†
BSA (M^2)	1.89 ± .08	1.88 ± 0.05
TBSB (%)	—	56.1 ± 5.3 (30–77.5)†
PBD (days)	—	8.7 ± 1.5 (5–16)†
% PBW	—	103.4 ± 3.14% (90.5–112.3)†

* Mean ± SEM

† Range.

BSA, body surface area; TBSB, total body surface burn; PBD, postburn day of study; % PBW, % preburn weight.

After injection of I-HIP, plasma samples were taken at 5, 10, 15, 20, 40, 50, 60, 70, 80, 100, and 120 minutes for counting in a gamma scintillation detector and determination of effective renal plasma flow.[9] Effective renal plasma flow was calculated as the clearance of I-HIP by fitting the plasma ^{131}I radioactivity to a biexponential expression of time after the dose $[a_1\exp(b_1 time) + a_2\exp(b_2 time)]$ and determining the dose of ^{131}I counted in dilution separately $[clearance = -dose/(a_1/b_1 + a_2/b_2)]$.

A ^{51}Cr-tagged red cell method using an f-cell correction of 0.87 and the peripheral hematocrit was used to estimate whole blood (Bvol) and plasma (Pvol) volumes from the red cell volume (RBCvol).[10,11] These volumes (measured in milliliters) were compared with those predicted as normal[10] on the basis of sex and body size (male subjects: $RBCvol = 1486\ BSA^2 - 4106\ BSA + 4514$; $Pvol = 995\ \exp(.6085\ BSA)$; female subjects: $RBCvol = 1167\ BSA - 479$; $Pvol = 1278\ BSA^{1.289}$; predicted Bvol was the sum of the predicted RBCvol and Pvol). If the mean observed/predicted ratio ± the 95% or 99% confidence interval of the mean for the patients did not overlap 1, the respective (0.05 or 0.01) significance level was determined. Formulas[12] for direct prediction of expected normal Bvol based on sex and body size yielded values very close to the sum of predicted RBCvol + Pvol and did not alter the results.

Cortisol, aldosterone, ADH, and ANF were determined by radioimmunoassay (RIA) at the Nichols Institute, San Juan Capistrano, California, where plasma renin activity (PRA, RIA of generated angiotensin I) and corticotrophin (ACTH, two-site immunoradiometry) were also determined. Hormone values were above the detectable limits except for ADH in five controls, in whom the ADH value was recorded as 1 pg/mL, the least detectable value.

Data were analyzed using BMDP software[13] (BMDP statistical software, Los Angeles, CA) on a Vax 3400 computer (Digital Equipment, Maynard, MA). The nonrectilinear regression (P3R) program was used to determine the parameters of the fit of plasma ^{131}I to time after injection of I-HIP. The P7D program was used to compare variables between burns and controls with the t test.

Results

As expected several hemodynamic variables differed significantly between the two groups in a manner consistent with the hyperdynamic response to injury (Table 2). The patients were tachycardic, with a widened pulse pressure. Flow variables (effective renal plasma flow and CI) were significantly increased in patients (Table 2).

TABLE 2. *Hemodynamic Variables*

Variable	Control	Patients
HR (bpm)	62 ± 6*	119 ± 3§
MBP (mmHg)	83 ± 3	77 ± 3
PP (mmHg)	45 ± 3	58 ± 4†
ERPF (mL/min/1.73 m^2)	525 ± 26	774 ± 96‡
CI (L/min/m^2)	[norm 2.3–4.1]	7.78 ± 0.52§

HR, heart rate; MBP, mean blood pressure; PP, pulse pressure; ERPF, effective renal plasma flow measure by ^{131}I Hippuran; CI, cardiac index.

* Mean ± SEM.

† $p < 0.05$; ‡ $p < 0.01$; § $p < 0.001$.

TABLE 3. *Total Blood Volume*

Patient	PBD	TBV (mL/kg)	Reference Normal Range (mL/kg)
1	5	51.14	60–80
1	12	55.64	60–80
2	7	56.90	60–80
3	10	68.34	60–80
4	6	46.00	55–75 (female)
4	10	53.37	55–75 (female)
5	16	61.61	60–80
6	11	47.11	60–80
7	6	63.97	60–80

The second measurement in patients 1 and 4 are not included in the data of Figure 1.

Abbreviations are defined in Tables 1 and 2.

The patients' RBC volumes measured by ^{51}Chromium-labeled red blood cells were significantly less than predicted. Plasma volumes were 100% of the predicted values based on body surface area, while total blood volumes were 81% of the mean predicted values (Fig. 1). Because the observed/predicted total blood volumes had a wide variance (95% confidence limits of this value range from 70% to 92% for this patient population), patient values were also compared to the laboratory reference normal range for blood volumes based on body weight. Three patients had total blood volume measurements that were within the reference normal range, while four patients (two measured twice) had total blood volumes that were less than the lower value of the normal range (Table 3).

Mean plasma sodium and osmolality were identical for the two groups (Table 4). Urine flow was significantly greater in control subjects, while urine osmolality was significantly greater in the patients, despite similar intravenous fluid administration rates. Free water clearance was 2.43 mL/min/1.73 m^2 in controls and −1.65 mL/min/1.73 m^2 in patients. Elevated urinary potassium concentrations and K + /Na + ratios were noted in the patients in association with slightly lower serum potassium levels and nondepressed urinary sodium values (Table 4).

Hormone values for patients and controls are tabulated in Table 5. AM cortisol levels were significantly greater in patients, as expected, while patient ACTH levels were not elevated. Plasma renin activity, ADH, and atrial natriuretic peptide levels were significantly greater in patients. Plasma aldosterone levels tended to be greater in patients but not significantly so when compared to the control population. Antidiuretic hormone levels were normal for the three patients with normal blood volumes and elevated for the four patients with decreased blood volumes ($p = 0.05$) (Table 6).

Discussion

Altered neural set points controlling the release of ADH and the renin-angiotensin-aldosterone axis have been invoked as an explanation for the elevated levels of these hormones, which are characteristic of the postresuscitative phase of burn care. Previous reports by Soroff et al.,[14] Collentine et al.,[15] Dolocek,[16] and Shirani et al.[1] have all assumed that the findings of significant sodium excretion and urine flow, hyponatremia in some patients, low or normal plasma osmolality, and elevated blood flow as

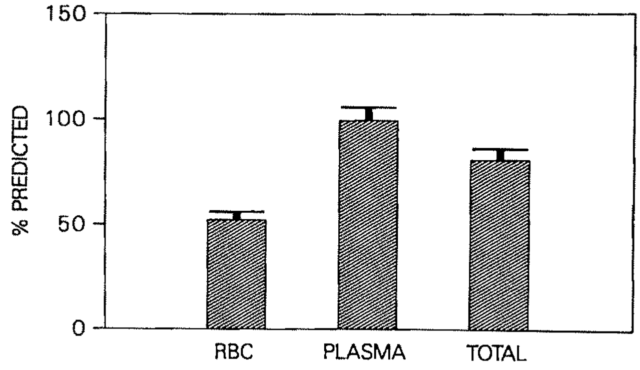

FIG. 1. The percentage of predicted red cell volume (RCV), plasma volume (PV), and total blood volume (TBV) for all patients is depicted. The RCV and TBV differs significantly from predicted values ($p < 0.01$), while calculated plasma volume was normal.

TABLE 4. *Serum and Urine Levels*

	Controls	Patients
Plasma Na$^+$ (mmol/L)	137.5 ± 0.8*	137.7 ± 1.5
Urine Na$^+$ (mmol/L)	39.1 ± 4.5	70.0 ± 22.4
Na$^+$ excretion (meq/hr/1.73 m^2)	11.5 ± 1.0	7.8 ± 3.5
Plasma K$^+$ (mmol/L)	4.52 ± .09	3.96 ± 0.12‡
Urine K$^+$ (mmol/L)	8.9 ± 0.9	52.3 ± 6.3§
Urine K$^+$/Na$^+$	0.24 ± 0.03	14.3 ± 9.2†
Plasma OSM (mosm/kg)	286 ± 1	286 ± 6
Urine OSM (mosm/kg)	160 ± 12	656 ± 45§
Urine output (mL/hr/1.73 m^2)	303 ± 19	87 ± 15§
FWCL (mL/min/1.73 m^2)	2.43 ± 0.3	−1.65 ± 0.15§
Intake (mL/hr)	250	284 ± 32

FWCL, free water clearance.

* Mean ± SEM.

† $p < 0.05$; ‡ $p < 0.01$; § $p < 0.001$.

TABLE 5. *Hormone Values*

Value	Controls	Patients
ACTH (pg/mL)	27.6 ± 5.7	13.0 ± 2.2
Cortisol (g/dL)	10.8 ± 1.1	22.6 ± 2.8‡
PRA (ng/mL/hr)	1.3 ± 0.3	28 ± 8‡
Aldosterone (ng/dL)	4.2 ± 1.4	11.7 ± 5.6
ADH (pg/mL)	1.2 ± 0.1	5.6 ± 2.5*
ANF (pg/mL)	78 ± 6	167 ± 34†

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

indexed by increased glomerular filtration rates and cardiac output are an indication of normal or increased blood volume. These findings, in combination with less than maximally dilute urine and elevated plasma levels of ADH, have led to the diagnosis of SIADH. The apparent dissociation of blood volume and flow indices documented in our patients indicates that the finding of increased flow may not support the assumption that a volume factor is absent in burn-induced SIADH, especially when plasma ADH and urine tonicity are high, even in the setting of a low plasma tonicity. Thus it is possible that if free water delivery is high enough, low blood volume-induced ADH secretion may promote hyponatremia, as seen in many burn patients. Although we did not test this hypothesis in our patients, the mean 20% decrement in total blood volume would suggest that the elevated levels of plasma ADH are an appropriate response in an attempt to restore blood volume.

Although hypotension and increased serum osmolality, by stimulation of stretch and osmo receptors, respectively, are the most potent stimulators of ADH release, modest decrements in blood volume also may cause appreciable pituitary release of this hormone. A 10% decrease in blood volume has been previously shown to result in a two- to threefold increase in plasma ADH levels.[17] In addition blood volume deficits of 10% to 15% are known to decrease the osmotic threshold for the release of ADH, although the linear relationship between plasma osmolality and plasma ADH levels is maintained. In our patients a 19% blood volume deficit resulted in a 4.5-fold increase in plasma ADH levels. The significantly decreased free water clearance and increased urine osmolality seen in our patients, as compared to the control population, document the expected influence of elevated ADH levels on the kidney.

Elevated plasma renin activity and aldosterone levels have been noted in burn patients during the postresuscitative phase.[1] As in the previously referenced ADH studies, blood volumes were not measured and the findings of elevated creatinine clearance and normal plasma tonicity were used as evidence for at least normal blood volume at the time of study. These findings, in concert with a normal plasma aldosterone decrease after a mild volume stimulus, were interpreted to mean that the renin-angiotensin-aldosterone system remained volume responsive in burn patients but that the elevated level of function occurred because of resetting of control mechanisms. The excess renin release was attributed, at least in part, to excess sympathetic activity, which occurs following burn injury and is also known to increase renin release.[18] In our patients plasma renin activity was significantly elevated compared to the control population. Plasma aldosterone levels, although elevated, were not statistically different from normal. The elevation in PRA in our patients is consistent with the anticipated increase in sympathetic activity in burn patients. It is possible that the one time measurement of fluctuating plasma aldosterone may not have been sufficient to reveal an elevated integral aldosterone level that may have been present in light of the elevated urinary $K+/Na+$ ratio. Nevertheless the relatively small aldosterone response to plasma renin activity has been described commonly in critically ill patients.[19] Our concurrent finding of increased atrial natriuretic factor, which is known to decrease aldosterone synthesis, may explain partially this finding.[6,20–22] Elevated urinary potassium concentrations and $K+/Na+$ ratios, despite slightly lower serum potassium and nondepressed urinary sodium in the patients, suggest an elevated aldosterone effect due, more likely, to a volume deficit than to sodium unavailability.

The finding of elevated atrial natriuretic factor levels does not fit entirely with our understanding of the normal stimuli for this cardiac hormone's release. Typically elevations in blood pressure and atrial distension secondary to blood volume excess are the stimuli that result in an increase in ANF release.[5] Neither of these mechanisms were operative in our patients. Recently it has been reported that, *in vitro*, elevated levels of ADH and angiotensin added to the media with freshly excised rat atria resulted in a significant increase in ANF release.[23] Thus the elevated ADH levels documented in our patients may result in an increased release of ANF. Elevated levels of ANF have been reported following thermal injury and resuscitation.[24] Other investigators[25] have reported a correlation between heart rate and ANF release that is independent of volume. The tachycardia documented in our patients thus may be partially responsible for the el-

TABLE 6. *Comparison of ADH Levels for Patients with Low and Normal Blood Volumes*

	Low	Normal
% Predicted TBV	75.8 ± 2.8	91.8 ± 2.7†
ADH level (pg/mL)	7.72 ± 2.5	1.97 ± 0.26*
n	6	3

TBV, total blood volume.
* $p = 0.05$; † $p < 0.01$.

evated levels of ANF, although this mechanism has recently been disputed.[26] This, in turn, may have a negative effect on aldosterone secretion. Atrial natriuretic factor appears to decrease aldosterone levels by inhibiting synthesis of this hormone in the adrenal glomerulosa cells.[20]

Except for the absence of hyponatremia, our patients are remarkably similar to those previously reported. Shirani et al.[1] studied nine burn patients in whom elevated ADH levels were documented. Those patients had hypertonic urine with a urine output of 2.7 L/day, a mean urine osmolality of 500 mosm/kg, and a mean urine sodium of 80 mmol/L (millimolar). Those values are quite comparable to those of the present study patients, who had a mean urine flow of 2.3 L/day, a urine osmolality of 656 mosm/kg, and a mean urine sodium of 70 mmol/L. The mean plasma ADH level of 6.8 pg/mL reported previously was very close to the levels documented in our patients (5.6 pg/mL). The only differences between the two patient populations were the serum sodium and osmolality: the former was 138 mmol/L in our patients compared to 130 mmol/L in the earlier study. The mean plasma osmolality of the patients in the earlier study was lower than in the patients in the present study (276 *versus* 286 mosm/kg). The serum sodium of 130 mmol/L was interpreted as being consistent with a free water excess indicative of increased blood volume. However those laboratory findings are also consistent with an intravascular volume deficit in association with a large intravascular sodium deficit, which may occur with excessive third space fluid losses and an osmotic diuresis prompted by urea; both circumstances occur following thermal injury.

It appears that the central problem in our cohort of patients involves mobilization of the edema fluid to the intravascular space. Five of the seven patients' weights were markedly more than their preburn weights on the day of study. In addition, because the total 24-hour intake surrounding the study period exceeded the estimated wound evaporative loss by at least maintenance fluid requirements in each patient, they were all considered to have received adequate replacement of ongoing fluid losses. Fluid administration rates were dictated by the patients' urinary output and serum sodium. In their management, decreasing urine outputs and increasing serum sodium levels were interpreted to be consistent with a contracting blood volume and resulted in an increase in the rate of relatively hypotonic intravenous fluid administration. Failure to adjust the rate was normally followed by an increase in serum BUN and creatinine levels and other signs of prerenal azotemia. After resuscitation shifts of water from the interstitial to the intravascular space normally occur secondary to differences in oncotic pressure, which normally favors water movement from the interstitial to intravascular space. Following burn injury and resuscitation, the intravascular colloid osmotic pressure is low, thus decreasing the net force that determines water movement. Whether artificially increasing plasma colloid osmotic pressure during this time period would improve edema mobilization cannot be answered from these data.

Findings of increased blood flow associated with a hyperdynamic circulation (elevated cardiac output) and increased organ flow (increased effective renal plasma flow, increased wound blood flow) have previously been interpreted to indicate a normal or supranormal blood volume. Our findings of increased flow in conjunction with modest decrements in total blood volume appear to be paradoxical. The reasons for the dissociation of flow and volume may lie in the neural peripheral vascular response to injury. The effects of the markedly elevated beta adrenergic activity,[18] which occurs following injury in association with what amounts to an effective arterial-venous shunt in the wound, may be enough to counterbalance the measured decrements in blood volume. Thus the decrease in peripheral vascular resistance typical of the flow phase of injury may serve to decrease afterload and raise the effective arterial capacity, which is underfilled at sites of hormone control.

The interpretation of blood volume measurements is complicated by various factors. First there is a difficulty in obtaining simultaneous measurements of RBC volume and plasma volume. The use of ^{51}chromium-labeled RBCs represents a well-standardized and accepted method for the measurement of RBC volume.[27,28] To obtain plasma volume estimates, one may either measure the space directly by the use of radiolabeled albumin or estimate it by the use of RBC volume and hematocrit levels. Use of labeled albumin in critically ill patients significantly overestimates plasma volumes due to the expanded volume of distribution for this molecule. Because of this plasma and total blood volumes in burned patients are usually estimated from the RBC volume and hematocrit measurements. Normal total blood volume has been reported to range from 60 to 80 mL/kg for men and 55 to 75 mL/kg for women. However, for comparison, we first expressed our measured patient values each as a percentage of the expected normal volume for that patient estimated from BSA according to previously reported regressions. As a group our patients had a total blood volume that was 81% of the predicted value. We then compared each individual patient to the reference range. Three patients had blood volumes within the normal reference range, while the remaining four (two measured twice) had blood volumes that were less than the expected range. The three patients with normal blood volumes had ADH levels that were not different from the normal controls. The patients with low blood volumes had elevated levels of ADH, the expected response based on blood volume.

It appears from our data that cardiac output and renal perfusion in burn patients may not reflect effective blood volume status as registered at hormonal control sites. The dissociation of organ flow and hormonal response necessitates simultaneous direct measurements of blood volume to elucidate factors other than resetting of central neural control mechanisms to explain hormonal changes in the flow phase of injury. Furthermore these data indicate that in burn patients increased blood flow does not depend on increased blood volume.

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DISCUSSION

DR. D. WILMORE (Boston, Massachusetts): The group at the US Army Institute of Surgical Research have had a long-term interest in fluid regulation and water and sodium balance in burn patients. This is another interesting and provocative paper that is a continuation of work in this area that has originated from this Institute.

There are a number of things that could be added to the paper that would help us with understanding, accounts of both studies performed in the normals and those investigations done in patients, and I would just like to mention a few of these points.

First normal individuals are usually standardized in terms of diet, fluid and salt intake, and in terms of exercise and environmental conditioning when renal function tests are done. Would the authors outline their standardization that was provided for their normal controls?

Patients also should be standardized in terms of fluid intake, salt intake, whether they were ventilated, if were they receiving enteral or parenteral feedings, and the type of medication they received. This information also would be quite helpful if it could be included in the manuscript.

The US Army Institute of Surgical Research reported in an earlier paper that patients do not have full colonization of burn wounds, and that overtime colonization occurs. Could it be that the bacteria on the wound's surface elicits factors that are vasoactive and over time could change blood volume regulation? We need to know more about the microbiology of these particular patients.

Of course the crux of the contention is that we can measure blood volume using this chromium label technique, and it is a very well-established technique, but it would be good to know what the variation in the measurement was if the technique were used in normal individuals and what the reproducibility of the measurement was with successive determinations from week after week measurements. Such data would give us a good deal of confidence concerning these measurement.

Moreover it may well be that in expressing blood volume, we should express it in terms other than kilogram body weight or body surface area. As you recall these patients were edematous, and we really do not know their "true" or actual body weight. Would it be better to measure total body water and express blood volume as a unit of lean body mass or to use bioelectrical impedance to express blood volume as a unit of lean body mass, correcting or adjusting for edema?

But if these patients were experiencing a functional volume deficit, would they respond normally to volume loading? Have the authors given blood to these patients and seen alterations in their vasoactive or salt-retaining hormones as blood is infused? It seems to me that that would be a much more dynamic type of functional measurement if it could be performed.

Finally in this modern age of biotechnology, could this whole presumed deficit be resolved by giving recombinant erythropoietin to stimulate blood volume mass, which is low in these patients?

This is an interesting and indeed a provocative paper, and as we learn more about the regulation of blood and fluid volume, I am sure that this new information will help us take better care of our critically ill patients.

DR. DONALD GANN (Baltimore, Maryland): I think this is a very important study that was very carefully executed. Part of what I have to say will echo some of Dr. Wilmore's comments, but I have a somewhat different perspective.

I also am grateful to the authors for sending me the manuscript, because it demands very careful examination. It is very meaty indeed.

I also am particularly grateful for this study, because when Dr. Shirani first suggested that inappropriate antidiuretic hormone (ADH) secretion was a feature of the burn patient, I discussed the paper and suggested that in fact it may be attributable to blood volume. Thus this is one of those rare occasions when one can actually suggest that somebody else do a study and see it carried out with the proper results. I probably should stop there.

I would note, however, that we have known since the landmark publication of Leaf and Mamby in 1950 that a small volume deficit can lead to sufficient persistent hypovolemia; so that normal subjects—in that case, Harvard medical students who were presumed normal—could drink themselves into water intoxication, implying persistent elevation of vasopressin.

In the early 1960s, Hastings, Wright, and I showed that in postoperative patients, the administration of saline could suppress vasopressin sufficiently to produce free water. That, in a less severely injured population, is the answer to Dr. Wilmore's question: namely, you can indeed turn off those hormones. Cross and others from my own laboratory, in a paper published about a year ago in the *Annals*, showed that in a group of postoperative cardiac surgical patients the counter-regulatory hormones can be suppressed by volume loading. Dr. Vaughan from Dr. Pruitt's group and others reported consistent elevation in cortisol in burn patients for as long as 30 days. This was a dominant feature of the severely injured patients.

Those observations that I have mentioned suggest to me several questions, perhaps the most important one of which is that mentioned by Dr. Wilmore: namely, what was the intake in the normal subjects as well as in the patients? The control subjects excreted in excess of 7 L of water a day. Assuming that they had normal bladder capacity and slept approximately 8 hours, their entire waking life was spent excreting large volumes of urine.

They also excreted in excess of 250 mEq sodium in a day, a very high sodium load, which probably explains their limited production of free water, but certainly does not suggest that they were eating a normal diet. Were they matched for the salt and water intake of the patients? I assume so, but this was not mentioned in the manuscript.

Does the decreased blood volume seen in the patients account for the prolonged elevation of cortisol? Cortisol, as we have shown, is probably the dominant hormone responsible for restitution of blood volume after production of an acute volume deficit.

And finally, and most important, if neither the hemodynamic status of the patient nor the urine output can adequately reflect the effective circulating volume, how do the authors propose to ascertain the adequacy of replacement in burn patients? This is particularly important because continued elevation in cortisol and in other substances responding to a volume deficit may be important mediators of continued immunosuppression and ultimately of sepsis in these highly susceptible patients.

DR. DAVID HERNDON (Galveston Texas): This fascinating paper demonstrates a remarkable, if not astounding, paradox. It really flies against traditional wisdom previously published by these authors. They demonstrate in patients with large burns at 8 days postburn that they are hypovolemic rather than being, as we previously had thought, hypervolemic.

Despite this demonstrated hypovolemia, these patients are markedly hyperdynamic and had increased cardiac indices and renal blood flow: another paradox. They have, as previously shown, antidiuretic hormone (ADH), cortisol, and renin levels that are markedly elevated. This has previously been attributed to inappropriate ADH production in response to burn stress, but by measuring red blood cell volume with an extremely useful chromium labeling technique, it is believed that these hormonal changes are normal responses to hypovolemia.

A further paradox, however, is the finding of the elevated atrial natriuretic factor (ANF) levels. This substance is primarily controlled by atrial stretch, and hypervolemia is its usual stimulus.

These findings are puzzling, and I have several questions. The plasma volume was calculated, but not measured. It was thought to be 100% of normal. The whole of the blood volume decrease was accounted for by a decrease in red blood cell volume. In most anemias, plasma volume reciprocally increases as blood cell volume decreases. I am wondering why this did not occur in this setting.

In this study the plasma volume was calculated from the hematocrit. Why did the authors not directly measure plasma volume with a dye or radioactive technique, as I believe they have in the past?

How confident are they in these calculations of plasma volume, on which so much of their hypotheses rest?

I too was curious as to why the urine output of their controls was 300 mL/hour per 1.73 meter square, whereas it was only 87 mL for the same indices in the patients. That is a marked discrepancy. How do the authors account for the massive urine output of their controls, where they allowed water, beer, or coffee *ad libitum?*

Is it conceivable, given these huge urine outputs in the controls, that they were volume expanded, which would affect the significance, of course, of the comparisons of renin, ANF, ADH, cortisol, and, most markedly, the clearances between the two groups?

Serum albumin or colloid osmotic pressure could clearly affect these results. These values were not provided in the manuscript, and I would be curious as to what they were.

Why did you not measure blood volume in your control patients? Their urine output would indicate they were hypervolemic, of course.

Because you measured cardiac index—Swan-Ganz catheters were clearly in—did pulmonary artery wedge pressures correlate with this demonstrated hypovolemia?

Have you changed your clinical practice since the prior publication of Shirani on this subject, in which urine outputs were higher in patients and the serum sodiums were 130, as opposed to the serum sodium of 138 in your current group? Are you fluid restricting patients now and making them relatively hypovolemic? Perhaps in the earlier study, patients were normovolemic and the postulative inappropriate ADH secretion could stand.

Finally the demonstrated hypovolemia is potentially deleterious. Vasoconstricton to some organs, perhaps to the gut, may be required to maintain a hyperdynamic state in the face of hypovolemia. This could result in bacterial or endotoxin translocation from the gut. Should we be more liberal, then, in fluid loading burn patients with red blood cells or colloid during the second week postburn to reverse this demonstrated hypovolemia with its potential deleterious effects?

DR. FRANCIS D. MOORE (Boston, Massachusetts): The conservation of body water is one of the most basic and primitive responses of all land-dwelling vertebrates. Now I have always been interested in the primitive nature of much of the response to surgery and to injury. These responses have Darwinian survival significance. Once upon a time, I wrote a carefully documented chapter on that subject for a surgical textbook. Although it has been—wisely, I am sure—withdrawn from that textbook, there is a message there that should not be forgotten.

So when we call antidiuretic hormone (ADH) secretion "inappropriate," we'd better be careful. "Inappropriate" to whom, for what, in whose judgment?

As you know, Verney, the originator of all this work, thought ADH was secreted in response to elevated osmolarity.

Leaf and Bartter then, in a brilliant series of experiments in humans, showed that given a clear choice between maintaining volume and maintaining osmolarity by ADH increases, the body would always prefer to restore volume. So Verney's idea had to be a little bit changed.

Also there are neural and other sensory pathways involved. David Hume, before he became a pioneer transplanter, was working in this field. We did a lot of work together on this subject. Ether anesthesia is a strong stimulus to ADH, as is morphine analgesia. A bleed with prompt retransfusion immediately stimulates ADH. Finally we discovered that the first few milliliters that were lost (even the venipuncture itself) would stimulate ADH secretion. High altitude and low PO_2 will increase ADH secretion. In fact some of the headache that is seen in people at high altitude is due to excess loading of water and salt in the body and a disproportionate representation in the cerebrospinal fluid.

So we have to be very, very careful before we call ADH "inappropriate." We have got to be careful before we assign it to some one cause such as tonicity or volume, when so many things can stimulate this water-saving survival response.

In young men going to bed at night, there is a strong antidiuresis. Sadly this is not seen in older men. Just why old men lose this God-given ability to concentrate their urine and lower their voiding volume at night, I have never been able to figure out. But it has only bothered me for a few years, so there is still hope—or is there?

Finally I would like to congratulate Basil and Dr. Mason and the team at Brooke. For some years I was a member of the Surgeon General's committee that inspected that unit, and our inspections were really quite long, because I wanted to learn everything that I could while I was there! You have been leaders in burn therapy for all these years, and so far as I know, care for the biggest load of severely burned patients in the United States. That has never dimmed your interest in discovering new things that would be helpful to everybody.

So I would like to congratulate you. But take it easy on that "inappropriate" business.

DR. W. G. CIOFFI (Closing discussion): Dr. Wilmore, your first question was about the standardization of the normal volunteers. They were admitted 1 day before study and were allowed food until 6 hours before their study. They were started on IV fluids (D5 in ¼ NS) at rates that we hoped would match the IV intake in the patients, and this resulted in some of the discrepancies noted by Dr. Herndon in terms of urine output and sodium excretion.

The patients were all receiving maintenance fluids, as well as hypotonic solutions to replace their estimated evaporative losses. This allowed approximately a 10% weight loss per day of their initial weight gain.

The patients were not infected. All patients were receiving enteral feedings at the time of the study, and their fluid management was as outlined. I do not have data on the bacterial density on their wounds; no patient, however, had a wound infection before or during the study.

We did not do the chromium label study on the normal volunteers. We do have data on normals, however, which are from our Nuclear Medicine Department, which performed this part of the study, and they have consistently shown patients who should be expected to be in the normal range to be so.

Your recommendation for expressing blood volume as an index of lean body mass is a good one, but we do not have the data available to do that.

The value of erythropoietin in these patients is not clear. Several papers presented last week at the American Burn Association demonstrated already high endogenous circulating levels of erythropoietin after injury. Whether or not the addition of exogenous erythropoietin will help to restore the red cell volume to normal is not clear.

Dr. Gann, I think I have answered your question as far as the intakes in the normal controls as compared with the patients. I agree that the deficit we saw in total blood volume may well be responsible for the prolonged elevation to cortisol levels.

You asked about what we could do to assess the adequacy of fluid replacement after resuscitation in patients, if indeed cardiac output or urine output are not going to be reliable indicators. I think we do have several things that we could follow in our second study, which we plan to undertake soon, in which we will attempt to volume load these patients, either with red cells or with saline to a normal blood volume, and we will look at cardiac output to see if it will decrease, as well as looking at O_2 consumption measured by indirect calorimetry.

Dr. Herndon, you noted the paradox of our increased atrial natriuretic factor levels Typically atrial distension has always been thought to be a stimulus for this hormone's release, but there are also significant data in the literature suggesting that elevated sympathetic tone, tachycardia, elevated vasopressin, and aldosterone levels also lead to hormone secretion.

It also should be noted that atrial natriuretic factor (ANF) also serves to down-regulate plasma renin activity, angiotensin, and aldosterone, and it may be the elevated ANF levels that were responsible for a lack of a consistent finding of elevated aldosterone.

We did not measure plasma volume. It has been done before. We chose not to do that, because we believe there is no reliable marker to use in these patients at a time when albumin flux is continuing across into the wound, and thus radiolabeled albumin or similar markers would not give us an accurate assessment of plasma volume.

It is true that anemic patients with decreased red cell volumes increase their plasma volume to bring their blood volume back to normal. But that happens over a prolonged time and most notably in chronic renal failure patients. That is a chronic effect, and I wouldn't expect it to occur over postburn days 6, 7, or 8.

Our albumin levels were 2.2 or less in the patients. We did not measure COP levels.

There was not a good correlation between wedge pressure readings and blood volume. The mean pulmonary artery wedge pressure (PAWP) was 9. Our clinical practice has not changed since Dr. Shirani's study of several years ago.

As far as the liberal use of red cells for volume replacement, I would recommend that over the use of plasma volume expanders. We must take into consideration a report that we published several years ago, however, which did show a relationship between red cell transfusion and subsequent risk of infection, and thus we have to consider that before charging ahead and infusing many units of red cells into these patients.

Dr. Moore, I would like to thank you for your comments and couldn't agree more that the term "inappropriate" is probably inappropriate in this meeting.

Hepatic Resection *Versus* Transplantation for Hepatocellular Carcinoma

SHUNZABURO IWATSUKI, M.D.,* THOMAS E. STARZL, M.D., PH.D.,* DANIEL G. SHEAHAN, M.B., M.SC.,†
ITSUO YOKOYAMA, M.D.,* ANTHONY J. DEMETRIS, M.D.,† SATURO TODO, M.D.,* ANDREAS G. TZAKIS, M.D.,*
DAVID H. VAN THIEL, M.D.,* BRIAN CARR, M.D., PH.D.,* RICHARD SELBY, M.D.,* and JUAN MADARIAGA, M.D.*

From the Departments of Surgery and Pathology,†*
University of Pittsburgh School of Medicine and the Veterans
Administration Medical Center, Pittsburgh, Pennsylvania

During the 10-year period (1980 to 1989), 76 patients with hepatocellular carcinoma (HCC) were treated by subtotal hepatic resection (HX) and 105 patients by orthotopic liver transplantation (TX) under cyclosporine-steroid therapy. Overall 1- to 5-year survival rates of the HX group were 71.1%, 55.0%, 47.2%, 37.2%, and 32.9%, respectively, and those of the TX group were 65.7%, 49.0%, 39.2%, 35.6%, and 35.6%, respectively. The survival rates after HX and after TX correlated well with pTNM stages and were similar in each stage between the two groups. However, when HCC was associated with cirrhosis of the liver, the survival rates after TX were significantly better than those after HX at each stage of pTNM classification. The tumor-recurrence rate was high both after HX (50%) and TX (43%), particularly in advanced stages of pTNM classification (60% or more). Twelve patients after HX and 13 patients after TX lived more than 5 years during this 10-year period. Fibrolamellar HCC and early stages of HCC were highly represented among the long-term survivors. Further improvement in survival rates depends on nonsurgical anti-cancer therapy before and/or after surgical removal of HCC.

LIVER TRANSPLANTATION HAS been widely accepted as a useful therapy for various advanced liver diseases.[1-3] However its role in the treatment of hepatobiliary malignancy still remains to be determined.[4-15] Our earlier reports[4-7] and those of others[8-11] have emphasized the high recurrence rate of hepatocellular carcinoma (HCC) in patients treated with liver transplantation, but they also recognized a handful of patients who were apparently cured of their malignancy by hepatic replacement. The same is true with conventional subtotal hepatic resection.[16-30]

In this study we compared the results obtained by liver transplantation to those achieved by subtotal hepatic resection among our own series of 181 patients with HCC according to the pTNM staging. The prognostic factors other than those included in pTNM staging were also examined for their significance.

Materials and Methods

During the 10-year period between 1980 and 1989, 76 patients with HCC were treated by subtotal hepatic resection (HX) and additional 105 patients by orthotopic liver transplantation (TX) under cyclosporine-steroid immunosuppressive therapy at the University of Colorado Health Sciences Center (1980), and at the University Health Center of Pittsburgh (1981 to 1989).

Liver transplantation was used if subtotal hepatic resection was not anatomically feasible due to extensive intrahepatic involvement of malignancy, or if the underlying liver disease and/or hepatic failure precluded this possibility. One third of the patients in the transplant group had anatomically unresectable HCC in the normal liver, one third had functionally unresectable HCC in the cirrhotic liver, and one third had misdiagnosed HCC in the failing liver. The extent of HCC was staged using the pTNM classification proposed by the International Union Against Cancer and the American Joint Committee on Cancer (Table 1).[31,32]

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Address reprint requests to Shunzaburo Iwatsuki, M.D., Department of Surgery, 3601 Fifth Ave., Falk Clinic 5C, Pittsburgh, PA 15213.

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TABLE 1. *pTNM Pathologic Classifications*

Classification			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1	N1	M0
	T2	N1	M0
	T3	N0, N1	M0
Stage IVA	T4	Any N	M0
Stage IVB	Any T	Any N	M1

T1: Solitary, ≤2 cm, without vascular invasion.
T2: Solitary, ≤2 cm, with vascular invasion.
Multiple, one lobe, ≤2 cm, without vascular invasion.
Solitary, >2 cm, without vascular invasion.
T3: Solitary, >2 cm, with vascular invasion.
Multiple, one lobe, >2 cm, with or without vascular invasion.
T4: Multiple, > one lobe.
Invasion of major branch of portal or hepatic veins,
N1: Regional
M1: Distant metastasis.

Subtotal Hepatic Resection Group (HX Group)

There were 76 patients in this group; 53 were male and 23 were female. The ages ranged from 9 to 86 years with a mean ± standard deviation (SD) of 51.4 ± 17.4 years. Seventeen of seventy-six patients had associated cirrhosis of the liver. Eight patients were chronic carriers of hepatitis B surface antigen (HBsAg), and three others had hepatitis B surface antibody and/or core antibody (HBsAb and/or HBcAb).

These 76 patients with HCC were stratified according to the pTNM classification and are shown in Table 2. Twelve of seventy-six HCCs were those of fibrolamellar variant (FL-HCC).

The follow-up periods of this group of patients ranged from 16 to 131 months, with a median follow-up of 53 months as of February 1, 1991.

Liver Transplantation Group (TX Group)

There were 105 patients in this group; 70 were male and 35 were female. The ages ranged from 3 to 69 years, with a mean ± SD of 43.5 ± 18.2 years. Seventy-one of one hundred five patients had associated cirrhosis of the liver. Twenty-three patients were chronic carriers of HBsAg and 11 others had HBsAb and/or HBcAb.

These 105 patients with HCC were stratified according to the pTNM classification and are shown in Table 3. Ten of one hundred five patients had FL-HCC.

The follow-up periods of this group of patients ranged from 16 to 131 months, with a median follow-up of 37 months as of February 1, 1991.

Prognostic Factors

The variables included in pTNM classification (Table 1) were examined individually for their influence on survival rates before grouping into the stages. Other variables examined were (1) associated cirrhosis, (2) HBsAg, (3) microscopic tumor margin, (4) shape of the tumor(s) (circumscribed *versus* infiltrative), and (5) fibrolamellar variant. All variables examined are listed in Table 7.

Statistical Analysis

Actuarial survival rates were calculated by the life-table method using the BMDP statistical software (University of California Press, Berkeley, CA). Statistical comparisons across different groups were made by the method of Mantel-Cox for univariate analysis. The Cox proportional hazards regression model was used to assess the relative prognostic importance of factors in predicting survival (multivariate analysis). Differences were considered significant if the probability value was less than 0.05.

Results

Survival Rates

Overall survival rates of the subtotal hepatic resection group (HX group) and those of the liver transplantation group (TX group) are shown in Figure 1 and Tables 4 and 5. There was no difference in the survival rates between the two groups.

In the HX group the survival rates of patients with FL-HCC were significantly ($p = 0.016$) higher than those of

TABLE 2. *pTNM Stages of 76 Patients with Hepatocellular Carcinoma Treated by Subtotal Hepatic Resection*

pTNM Stage	Number of Patients	Number of Patients	
	Total HCC	Non-FL	FL
I	0	0	0
II	19	16	3
III	25	18	7
IV-A	32	30	2
Total	76	64	12

HCC, hepatocellular carcinoma; FL, fibrolamellar HCC.

TABLE 3 *pTNM Stages of 105 Patients with Hepatocellular Carcinoma Treated by Liver Transplantation*

pTNM Stage	Number of Patients	Number of Patients	
	Total HCC	Non-FL	FL
I	4	4	0
II	19	17	2
III	23	23	0
IV-A	59	51	8
Total	105	95	10

HCC, hepatocellular carcinoma; FL, fibrolamellar HCC.

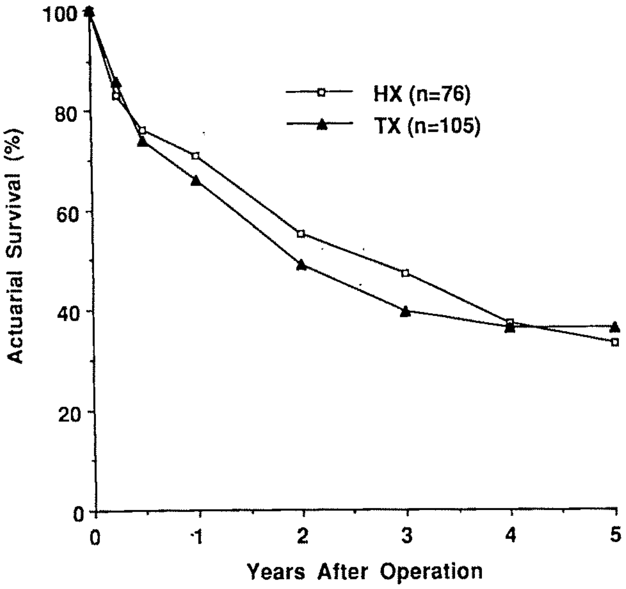

FIG. 1. Overall survival rates of patients with hepatocellular carcinoma. HX, subtotal hepatic resection; TX, liver transplantation.

patients with nonfibrolamellar hepatocellular carcinoma (non–FL-HCC) (Table 4). In the TX group, however, the survival rates were quite similar between them (Table 5) because 8 of the 10 patients were in stage IV-A (Table 3). The survival rates of patients with FL-HCC tended to be higher in the HX group than in the TX group, but the difference was not statistically significant (Tables 4 and 5).

When HCC was associated with cirrhosis of the liver, the survival rates were significantly ($p = 0.001$) lower than those without cirrhosis in the HX group (Table 4), but the survival rates were similar in the TX group (Table 5). The survival rates of the HX group were significantly ($p = 0.02$) lower than those of the TX group when HCC was associated with cirrhosis of the liver, but they were similar when HCC was not associated with cirrhosis (Tables 4 and 5). There was no 4-year survivor after subtotal hepatic resection (HX) among the patients with HCC in the cirrhotic liver, but the 5-year survival rate after transplantation (TX) was 40.7%.

The overall survival rates were stratified by the pTNM stages and are shown in Tables 4 and 5. Both in the HX group and in the TX group the pTNM stages correlated well with the survival rates. In both groups the survival rates of stage IV-A patients were significantly ($p < 0.05$) lower than those of other stages. There was no statistically significant difference in the survival rates when they were compared in the same pTNM stages between the HX group and the TX group (Table 4 and 5). However, when the survival rates of patients with HCC in the cirrhotic liver were compared in the same stages, the survival rates of the TX group were significantly ($p < 0.05$) better than those of the HX group (Table 6). The difference was most striking in stage III.

Prognostic Factors

Univariate analyses of five factors included in the pTNM classification and six others were performed by the Mantel-Cox test and the results were summarized in Table 7, with mean survival rates in months. The poor prognostic factors of statistical significance both in the

TABLE 4. *Survival Rates After Subtotal Hepatic Resection for Hepatocellular Carcinoma*

	3 months	6 months	1 year	2 years	3 years	4 years	5 years
Total HCC (n = 76)	82.9% (63)	77.6% (59)	71.1% (54)	55.0% (36)	47.2% (26)	37.2% (17)	32.9% (12)
FL-HCC (n = 12)	100% (12)	100% (12)	100% (12)	83.3% (9)	83.3% (9)	64.8% (6)	64.8% (5)
Non–FL-HCC (n = 64)	79.7% (51)	73.4% (47)	65.6% (42)	49.7% (27)	40.2% (17)	32.1% (11)	26.3% (7)
Cirrhosis (n = 17)	64.7% (11)	47.1% (8)	35.3% (6)	23.5% (4)	5.9% (1)	0 (0)	— —
Noncirrhosis (n = 59)	88.1% (52)	86.4% (51)	81.4% (48)	64.3% (32)	60.2% (25)	49.5% (17)	43.7% (12)
TNM Stage I (n = 0)							
TNM Stage II (n = 19)	100% (18)	100% (18)	100% (18)	84.2% (15)	78.6% (13)	58.2% (8)	43.7% (4)
TNM Stage III (n = 25)	80.0% (20)	80.0% (20)	76.0% (19)	68.0% (14)	53.4% (9)	46.8% (6)	46.8% (5)
TNM Stage IV-A (n = 32)	78.1% (25)	65.6% (21)	53.1% (17)	26.9% (7)	22.4% (4)	16.8% (3)	16.8% (3)

TABLE 5. *Survival Rates After Liver Transplantation for Hepatocellular Carcinoma*

	3 months	6 months	1 year	2 years	3 years	4 years	5 years
Total HCC (n = 105)	85.7% (90)	74.3% (78)	65.7% (69)	49.0% (45)	39.2% (26)	35.6% (16)	35.6% (13)
FL-HCC (n = 10)	90.0% (9)	90.0% (9)	80.0% (8)	70.0% (2)	50.0% (4)	37.5% (3)	37.5% (3)
Non–FL-HCC (n = 95)	85.3% (81)	72.6% (69)	64.2% (61)	46.8% (38)	38.3% (22)	36.5% (13)	36.5% (10)
Cirrhosis (n = 71)	84.5% (60)	71.8% (51)	63.4% (45)	48.6% (28)	42.9% (21)	40.7% (12)	40.7% (10)
Noncirrhosis (n = 34)	88.2% (30)	79.4% (27)	70.6% (24)	50.0% (16)	32.5% (6)	26.0% (4)	26.0% (3)
TNM Stage I (n = 4)	75.0% (3)	75.0% (3)	75.0% (3)	75.0% (3)	75.0% (2)	75.0% (2)	75.0% (2)
TNM Stage II (n = 19)	84.2% (16)	84.2% (16)	79.0% (15)	68.4% (12)	68.4% (12)	68.4% (8)	68.4% (5)
TNM STage III (n = 23)	87.0% (20)	78.3% (18)	78.3% (18)	59.8% (11)	59.8% (8)	52.3% (4)	52.3% (4)
TNM Stage IV-A (n = 59)	86.4% (51)	69.5% (41)	55.9% (33)	26.6% (15)	16.3% (4)	10.9% (2)	10.9% (2)

HX group and in the TX group were (1) multiple gross tumors, (2) vascular invasion, (3) advanced pTNM stages, (4) positive surgical margin, and (5) infiltrative shape of tumor.

The tumor size of more than 2 cm was a significantly poor prognostic factor in the TX group, but it could not be examined in the HX group because there was no tumor measuring 2 cm or less in this group. Bilobar involvement of tumor and lymph node metastasis were significantly poor prognostic factors in the TX group, but they were not significant in the HX group. Fibrolamellar histology was a significantly good prognostic factor in the HX group. The fibrolamellar patients had a higher survival rate in the TX group for the first 3 years, but this factor was not statistically significant because 8 of the 10 patients with this tumor were classified as stage IV-A. Associated cir-

TABLE 6. *Survival of Patients with Hepatocellular Carcinoma in the Cirrhotic Liver*

	3 months	6 months	1 year	2 years	3 years	4 years	5 years
HX Group							
Stage I (n = 0)							
Stage II (n = 2)	100% (2)	100% (2)	100% (2)	50 0% (1)	50.0% (1)	0% (0)	
Stage III (n = 5)	60.0% (3)	60.0% (3)	40.0% (2)	40 0% (2)	0% (0)		
Stage IV-A (n = 10)	60.0% (6)	30.0% (3)	20.0% (2)	0% (0)			
TX Group							
Stage I (n = 4)	75.0% (3)	75.0% (3)	75.0% (3)	75.0% (3)	75.0% (2)	75.0% (2)	75.0% (2)
Stage II (n = 16)	81.3% (13)	81.3% (13)	81.3% (13)	75.0% (11)	75.0% (11)	75.0% (7)	75.0% (5)
Stage III (n = 19)	89.5% (17)	79.0% (15)	79.0% (15)	56.1% (8)	56.1% (7)	48.1% (3)	48.1% (3)
Stage IV-A (n = 32)	84.4% (27)	62.5% (20)	43.8% (14)	26.2% (6)	0% (0)		

TABLE 7. *Univariate Analysis of Prognostic Factors (Mean Survival in Months [± SE])*

Factor	Subtotal Hepatic Resection	Liver Transplantation
Size of Tumor		p = 0.0241
≤2 cm	(n = 0)	76.4 ± 14.8 (n = 12)
>2 cm	51.7 ± 6.6 (n = 76)	40.4 ± 5.2 (n = 93)
Number of Gross Tumor	p = 0.0004	p = 0.0143
Single	65.5 ± 8.5 (n = 46)	68.6 ± 10.3 (n = 29)
Multiple	25.1 ± 6.1 (n = 30)	37.9 ± 5.5 (n = 76)
Lobar Involvement	p = 0.2916	p = 0.0003
Unilobar	41.3 ± 7.8 (n = 44)	66.1 ± 7.8 (n = 51)
Bilobar	60.2 ± 9.7 (n = 32)*	28.0 ± 5.2 (n = 54)
Vascular Invasion	p = 0.0000	p = 0.0002
None (V0)	67.1 ± 11.6 (n = 24)	73.2 ± 10.1 (n = 30)
Microscopic (V1)	51.8 ± 9.7 (n = 30)	43.3 ± 7.1 (n = 37)
Macroscopic (V2)	28.1 ± 9.2 (n = 22)	21.6 ± 5.6 (n = 38)
Lymph Node Metastasis	p = 0.6188	p = 0.0054
Absent (N0)	52.7 ± 6.8 (n = 72)	49.7 ± 5.4 (n = 96)
Present (N1)	28.0 ± 7.2 (n = 4)*	10.5 ± 3.7 (n = 9)
pTNM Stage	p = 0.0069	p = 0.0015
I	(n = 0)	81.1 ± 23.2 (n = 4)
II	64.0 ± 12.6 (n = 19)	70.0 ± 13.2 (n = 19)
III	57.0 ± 10.6 (n = 25)	53.7 ± 9.6 (n = 23)
IV-A	34.0 ± 8.7 (n = 32)	26.5 ± 5.3 (n = 59)
Histological Type	p = 0.0277	p = 0.7023
Fibrolamellar	84.9 ± 15.8 (n = 12)	51.4 ± 14.4 (n = 10)
Nonfibrolamellar	42.9 ± 6.5 (n = 64)	47.5 ± 5.5 (n = 95)
Associated Cirrhosis	p = 0.0000	p = 0.6450
Present	11.4 ± 3.0 (n = 17)*	50.0 ± 6.4 (n = 71)
Absent	64.6 ± 7.9 (n = 59)	39.0 ± 7.6 (n = 34)
Microscopic Margin	p = 0.0293	p = 0.0042
Positive	29.3 ± 11.2 (n = 14)*	10.7 ± 3.9 (n = 9)
Negative	57.7 ± 7.6 (n = 62)	49.9 ± 5.5 (n = 96)
HBsAg	p = 0.0097	p = 0.3183
Positive	17.1 ± 6.8 (n = 8)	30.4 ± 6.3 (n = 23)
Negative	56.9 ± 7.2 (n = 68)	49.6 ± 5.9 (n = 82)
Size of Uninodular Tumor	p = 0.1423	p = 0.0034
≤5 cm	26.0 ± 7.2 (n = 6)*	89.6 ± 12.4 (n = 18)
>5 cm	69.4 ± 9.1 (n = 40)*	28.1 ± 9.8 (n = 11)
Shape of Tumor	p = 0.0000	p = 0.0010
Circumscribed	63.1 ± 8.0 (n = 58)	57.2 ± 6.4 (n = 70)
Infiltrative	17.3 ± 5.4 (n = 18)	21.8 ± 7.5 (n = 32)

* The difference between the HX group and the TX group is statistically significant ($p < 0.05$).

SE, standard error.

rhosis of the liver and chronic HBsAg carrier status were significantly poor prognostic factors in the HX group but they were not significant in the TX group.

Multivariate analysis of prognostic factors that reached statistical significance in univariate analysis ($p < 0.05$) revealed that associated cirrhosis and infiltrative shape of tumor were independently significant factors of poor survival in the HX group. In the TX group, bilobar involvement, microscopically positive tumor margin, lymph node metastasis, and vascular invasion were independently associated with poor survival rates.

Tumor Recurrence and Cause of Death

The recurrence of HCC was confirmed in 38 (50%) of the 76 patients in the HX group and in 45 (42.9%) of the 105 patients in the TX group during the follow-up period. Although overall incidence of tumor recurrence was similar for the HX group and the TX group, the HCCs of stages II and III recurred more frequently ($p = 0.003$) in the HX group than in the TX group (Table 8). The incidence of tumor recurrence in stage IV-A was extremely high: 59.4% in the HX group and 67.8% in the TX group.

Forty-eight (63.2%) of the 76 patients in the HX group and 67 (63.8%) of the 105 patients in the TX group died during the follow-up period. Approximately two thirds of deaths were directly or indirectly related to the tumor recurrence, both in the HX group and the TX group. It is worth noting that tumor-related death among patients with HCC of stages II and III was significantly ($p = 0.013$) more frequent in the HX group than in Tx group (Table 8). Tumor-related death was quite frequent among patients with stage IV-A tumor both in the HX group (53.1%) and the TX group (64.4%) (Table 8).

Approximately one fifth of the patients in the HX group and in the TX group died of various complications of hepatic resection or transplantation that were not related to tumor recurrence (Table 8).

Five-year Survivors

There were 25 patients who survived for 5 years: 12 in the HX group and 13 in the TX group (Table 9). None of the twelve 5-year survivors in the HX group had associated cirrhosis of the liver, but 10 of the 13 5-year survivors in the TX group had HCCs that developed in the cirrhotic liver. Five of the twelve patients in the HX group and 3 of the 13 patients in the TX group had FL-HCC, which was highly represented among the long-term survivors.

Of the 25 patients surviving 5 years, 2 patients had stage I tumors, 9 patients each had stage II or stage III tumors, and 5 patients had stage IV-A tumors. Three of the five patients with stage IV-A tumors had FL-HCC and the remaining two patients had nonfibrolamellar HCC, both of whom were in the HX group. There was no 5-year survivor in the TX group who had nonfibrolamellar HCC (non–FL-HCC) of stage IV-A disease.

Discussion

The results of various therapies for HCC have rarely been reported using a universal staging system such as the TNM classification.[11,14,33] Thus the sensible comparisons of results among various therapies and among different reports have been very difficult. To compare our results of subtotal hepatic resection for HCC to those of liver transplantation, our patients were stratified according to their pTNM stage.[31,32] As shown in Tables 4 to 7, the pTNM staging has proved useful in predicting the survival rates after hepatic resection ($p = 0.0069$) and liver transplantation ($p = 0.0015$). Although all of the variables included in the TNM staging are significant prognostic factors (Table 7), the multivariate analysis of these factors among our patients revealed that macroscopic vascular invasion, lymph node metastasis, and bilobar distribution of the tumor(s) were independently significant among them in predicting the survival rates.

The factors other than those included in the TNM classification were also valuable in predicting prognosis of HCC (Table 7) as reported by others.[18–30] Our study confirms that associated cirrhosis of the liver and infiltrative shape of the tumor were independently significant in predicting the poor survival rates after subtotal hepatic resection and liver transplantation. Although the survival rates of patients with HCC were significantly lower than those without malignancy after liver transplantation,[1–11] they were quite similar to those after subtotal hepatic resection in each stage of the pTNM classification (Tables 4 and 5). However, when the patients had HCC in the cirrhotic liver, the survival rates after liver transplantation were significantly higher than those after subtotal hepatic resection in each stage (Table 6).

Our overall survival rates after subtotal hepatic resection for HCC (67.1% at 1 year, 47.2% at 3 years, and 32.9%

TABLE 8. *Tumor Recurrence and Causes of Death*

	Number of Patients		
	Recurrence of Tumor	Death With Tumor	Death Without Tumor
HX Group			
Stage II (n = 19)	10 (52.6%)	8 (42.1%)	2 (10.5%)
Stage III (n = 25)	9 (36.0%)	7 (28.0%)	6 (24.0%)
Stage IV-A (n = 32)	19 (59.4%)	17 (53.1%)	7 (21.9%)
Total (n = 76)	38 (50.0%)	32 (42.1%)	15 (19.7%)
TX Group			
Stage I (n = 4)	0 (0%)	0 (0%)	1 (25.0%)
Stage II (n = 19)	1 (5.3%)	1 (5.3%)	6 (31.6%)
Stage III (n = 23)	4 (17.4%)	3 (13.0%)	8 (24.8%)
Stage IV-A (n = 59)	40 (67.8%)	38 (64.4%)	10 (16.9%)
Total (n = 105)	45 (42.9%)	42 (40%)	25 (23.8%)

TABLE 9. *List of 25 Five-Year Survivors (12 in HX group and 13 in TX group)*

	Age/Sex	Histology	Cirrhosis	TNM Stage	Recurrence	Survival
HX Group						
1	9/M	FL	NO	II	No	Alive after 11 years
2	63/M	NON-FL	NO	IV-A	No	Alive after 10 years
3	43/F	NON-FL	NO	III	No	Alive after 9 years
4	14/M	FL	NO	III	No	Alive after 8 years
5	40/F	NON-FL	NO	IV-A	Yes	Alive after 7 years
6	39/M	FL	NO	III	Yes	Died after 7 years
7	33/M	FL	NO	IV-A	No	Alive after 7 years
8	40/M	FL	NO	III	No	Alive after 7 years
9	41/F	NON-FL	NO	III	No	Alive after 6 years
10	65/F	NON-FL	NO	II	Yes	Died after 6 years
11	50/M	NON-FL	NO	II	No	Alive after 5 years
12	62/M	NON-FL	NO	II	No	Alive after 5 years
TX Group						
13	26/M	FL	NO	IV-A	No	Alive after 9 years
14	47/F	NON-FL	YES	II	No	Alive after 9 years
15	3/F	NON-FL	YES	II	No	Died after 7 years
16	7/F	NON-FL	YES	I	No	Alive after 8 years
17	22/F	NON-FL	YES	III	No	Alive after 8 years
18	23/F	FL	NO	IV-A	Yes	Died after 6 years
19	4/F	NON-FL	YES	I	No	Alive after 7 years
20	8/F	NON-FL	YES	II	No	Alive after 6 years
21	3/F	NON-FL	YES	III	No	Alive after 6 years
22	9/M	NON-FL	YES	II	No	Alive after 6 years
23	48/F	NON-FL	YES	III	No	Alive after 6 years
24	42/M	FL	YES	II	No	Alive after 5 years
25	33/F	NON-FL	NO	III	No	Died after 5 years

at 5 years) were similar to or even slightly better than those reported by others.[17–30] However our survival rates after hepatic resection for HCC in the cirrhotic liver were significantly lower than those reported from Asia, where early stages of HCC were removed from the cirrhotic liver by limited hepatic resection.[21–30] Our overall survival rates after liver transplantation for HCC in the cirrhotic liver (63.4% at 1 year, 42.9% at 3 years, and 40.7% at 5 years) were higher than those after hepatic resection for HCC in Asia.[21–30] Our survival rates after liver transplantation for HCC Of Stages I and II (early stages) in the cirrhotic liver were 80% at 1 year and 75% at 3 and 5 years (Table 6). A meaningful comparison could not be made in our own patients because there were only two patients with HCC of stage II in the cirrhotic liver who underwent subtotal hepatic resection. However these survival rates were similar to or even better than those after hepatic resection for most favorable lesions in Asia,[26–30] although the hepatic functions were worse in the liver transplantation group than in the resection group.

It is impractical and even fraudulent to recommend that all HCCs in the cirrhotic liver should be treated by liver transplantation. It is equally incorrect to exclude the patients with HCC from liver transplantation when the malignancy is confined to the liver. The treatment of choice for HCC confined to the liver is subtotal hepatic resection when it is anatomically and functionally feasible. Liver transplantation is the treatment of choice for HCC confined to the liver when the hepatic functions are poor and/or the HCC cannot be removed by subtotal hepatic resection.

The HCC recurred in one half of the patients after hepatic resection and liver transplantation (Table 8). In the HCCs of stages II and III, the tumor recurrence was significantly higher after hepatic resection than liver transplantation. The fact may indicate either that the staging was less accurate in the hepatic resection group because only a removed part of the liver could be examined pathologically or that new HCC developed in the retained portion of the liver after hepatic resection. Nevertheless a relatively low incidence of tumor recurrence after liver transplantation for HCC of stages II and III were encouraging. On the other hand, two thirds of HCCs in stage IV-A recurred after hepatic resection and liver transplantation (Table 8).

Further improvement in survival rates will be achieved in some degree by reducing the incidence of early death from surgical complications, but the major progress will depend on nonsurgical anti-cancer therapy. For this series of our patients, organized plans of anti-cancer therapy before and/or after surgery were not available. Because the introduction of a novel immunosuppressive drug, FK 506, to our liver transplantation in mid 1989,[34,35] we have been exploring the possible beneficial effects of neoadjuvant and adjuvant therapy for primary hepatobiliary malignancy. The improvement may be expected with this approach, particularly in HCCs of stages II and III after hepatic resection and in HCCs of stage IV-A after hepatic resection and liver transplantation, in which the tumor recurrence is frequent.

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DISCUSSION

DR. RONALD W. BUSUTTIL (Los Angeles, California): Dr. Iwatsuki and colleagues have presented a comparative analysis of subtotal hepatic resection *versus* liver transplantation for hepatocellular carcinoma according to the TNM (tumor, nodes, and metastases) classification. Additionally they have evaluated other prognostic factors that might influence the outcome of these patients.

These data strongly support the tenet that liver transplantation plays an important role in the treatment of selected patients with hepatocellular carcinoma that is confined to the liver and is not amenable to subtotal resection. Moreover transplantation is superior to resection in patients with cirrhosis, because in this condition the tumor is most often multifocal.

To put this paper into perspective, however, I believe it is important to view it *vis-a-vis* a collective series of 452 patients treated with liver transplantation for hepatocellular carcinoma that has been previously reported.

May I have that slide, please? As shown here, the 2- and 3-year survival in this large group of patients is approximately 30%, and the 5-year survival is between 20% and 25%. The data presented today by Dr. Iwatsuki represents a measurable improvement over this, with a 40% to 50% survival at 2 to 3 years and a 36% survival at 5 years.

What factors have accounted for this benefit? The first that comes to mind is that all of the cases reported today were done in a modern area of liver replacement in which cyclosporine was the mainstay of immunosuppression, and many of the technical and management refinements of liver transplantation have been codified. Patient selection also could play a role.

This report includes a series of tumors that may have a more favorable prognosis: namely, fibrolammelar variant, 10 cases, although as mentioned, several of these were of the stage 4A variety; and early lesions, TNM1, stage 1, four cases; and TM stage 2, 19 cases. Although the former group, fibrolammellar, did not show a statistically improved survival, a trend in this direction certainly is apparent at 2 to 3 years. Moreover, stage 1 and stage 2 lesions behaved similarly to incidental hepatocellular tumors, which are known to have an exceptionally good prognosis after transplantation.

Notwithstanding the above, the results reported with TNM stage 3 clearly represent an improvement over previous reports. In fact, 5-year survival in this group was 47%, which contrasts with a recent paper by Ring et al that reports no patient survival beyond 1 year in the presence of lymph node involvement.

The reason for this difference is not apparent, and thus I would like to ask: How many of the stage 3 lesions in the Pittsburgh group were T3 N0; in other words, how many had no lymph node involvement?

Finally it is unlikely that we can achieve much better results from surgery alone. In our program all patients with tumors who are transplant candidates are enrolled in an adjuvant trial of continuous 5-fluorouracil, cisplatin, combination adriamycin therapy for 6 months. Other centers have similar protocols. Results up to 3 years have been encouraging. Hepatitis-B-infected patients, however, have a high incidence of tumor recurrence, which was not reported in the Pittsburgh series.

Dr. Iwatsuki, you alluded to the use of adjuvant therapy in your manuscript. Do you have any further information on this, and how it specifically impacts on stage 3 and stage 4A tumors?

DR. HENRI BISMUTH (Villejuif, France): I agree with the general conclusion of the paper, that is, the superiority of transplantation when compared with resection, but there are several points I would like to raise.

Over the last 10 years, we have treated 115 patients with cirrhosis and hepatocellular carcinoma (HCC), 60 by resection and 55 by orthotopic transplantation. We have excluded from this transplant group tumors that were incidentally discovered at subsequent histologic examination so as only to compare the two groups in which tumor was identified preoperatively.

With respect to the results, we obtained broadly similar figures to those of Dr. Iwatsuki and the Pittsburg group when we examined the resection or transplant group as a whole. That is the 3-year survival was slightly better for transplantation (61%) than for resection (50%), but was much better, 54% and 24% respectively, when comparing survival without recurrence.

I would like to focus on two further points. When we compared our results for transplantation and resection, differences became highly significant when looking at single lesions: 74% and 25% 3-year survival without, respectively; and also for lesions less than 3 cm in size: 65% and 12%, respectively, again recurrence free at 3 years.

These clear advantages for transplantation are considerably reduced when we looked at lesions that were multiple or more than 3 cm in size.

So the point may be made by looking at the best and worse cases in which transplantation was performed in patients with less than three nodules all less than 3 cm in size or more than three nodules where one or more was more than 3 cm in size. Here the 3-year survival without recurrence is 93% and 10%, respectively. Thus in the latter group, the survival is comparable for secondaries and for HCC, in which people question whether we should transplant at all.

Although it may be unwise to extrapolate results from European patients, with HCC mainly occurring against a background of posthepatitic (B or C) cirrhosis, to those in America, I would be interested in knowing if Dr. Iwatsuki's large experience has led him to notice anything similar, especially in view of the fact that our conclusion—that we should be transplanting patients that we *could* resect and not transplanting those we cannot resect—superficially seems rather different from his own.

DR. SHUNZABURO IWATSUKI (Closing discussion): Answering Dr. Busuttil's question first, we have only four patients with lymph node positive in stage 3. In Hanover group, I believe that they had only six patients in stage 3, and that their survival is lower than ours. There are heterogenous patients in stage 3; lymph node positive and lymph node negative. TNM stage is useful, but if you read the rules of staging, there are still a lot of defects in the staging.

But in the past, we always presented the data of liver transplantation or hepatic resection without using any common ground of classification. The incidental tumor in Japan may be less than 2 cm, but in the United States, even a 5-cm lesion can be missed.

And with regard to adjuvant chemotherapy, we have been using a new adjuvant chemotherapy for hepatomas. We lose some patients because of the complications of chemotherapy, but we may gain some in long-term survival. The results are not in yet.

Answering Dr. Bismuth's questions of single tumor of less than 5 cm. If you treat this with liver transplantation, the survival rate is good. But if one can do the resection safely, there is no reason to transplant. There is a shortage of organs, and I think first choice of treatment for hepatoma or any tumor of the liver is subtotal resection.

Of course if we do the liver transplantation for a small tumor, the results are better, but there is no reason to do that. Liver resection can achieve the same result if the patient can tolerate the resection. The patients with a small tumor and with poor liver function tests should have transplants, not for cancer, but for the hepatic insufficiency.

Primary and Recurrent Crohn's Disease

Experience With 1379 Patients

FABRIZIO MICHELASSI, M.D., F.A.C.S.,* TOMMASO BALESTRACCI, M.D.,*
RICK CHAPPELL, PH.D.,† and GEORGE E. BLOCK, M.D., F.A.C.S.*

Between 1970 and 1988, 1379 patients with Crohn's disease were treated at the University of Chicago. Of these, 639 (mean age, 32.5 years; 322 men, 317 women) required at least one surgical procedure. The most common indications for operation were failure of medical treatment (n = 215, 33%), presence of a fistula (n = 154, 24%), and bowel obstruction (n = 141, 22%). A fistula was the most common intraoperative Crohn's-related complication. In 582 patients (92%), a resection was necessary, with primary anastomosis in 416 (65%), a temporary stoma in 124 (20%), and a permanent stoma in 42 (7%). The remaining 57 patients underwent diverse procedures (stricturoplasty, bypass, and so on). Two patients (0.3%) died. Follow-up data was obtained in 95%. One hundred eighteen patients developed recurrence requiring reoperation. The recurrence rate was 20% at 5 years and 34% at 10 years. The recurrence involved a permanent stoma or a previous anastomosis in 62 patients (afferent limb in 46, efferent in 16). In the 391 patients without previous surgery for Crohn's disease, a covariate analysis was performed to determine those variables significantly associated with recurrence. Variables included demographic data, findings at operation, surgical procedures, and histopathologic characteristics. The analysis revealed that the number of sites involved was the only variable that was significantly associated with the intra-abdominal recurrence rate ($p < 0.001$). The annualized risk of recurrence was 1.6% for patients with single-site involvement and 4% for those with multiple-site involvement. Perineal disease was associated with a significantly higher risk of local recurrence than any other site ($p < 0.02$). A subanalysis of 236 patients with single-site involvement but no previous operation allowed us to study the influence of site on indications for surgery and type of operative procedure. Failure of medical treatment was the most common indication for all sites. In contrast the site involved influenced the procedure: resection and primary anastomosis was feasible in 88% of jejunoileal and terminal ileal cases and a temporary ileostomy was necessary in only 12%. No patients with small bowel localization required a permanent stoma. A resection with primary anastomosis was feasible in only 32% of patients with colonic disease. The remaining two thirds of patients required either a temporary or a permanent stoma. It is concluded that multisite involvement is associated with 2.5 times the rate of recurrence of single-site disease, while the presence of perineal disease has a significantly higher incidence of local recurrence. Colonic disease carries a fivefold greater risk of stoma placement than other sites.

From the Departments of Surgery and Statistics,†*
University of Chicago, Chicago, Illinois

SURGEONS AND PHYSICIANS have sought to improve their ability to identify groups of patients at high risk for recurrence of Crohn's disease. Variables representing demographic, clinical, pathologic, and therapeutic characteristics have been analyzed to identify factors that might correlate with recurrence. Most of these studies have analyzed these characteristics through the use of univariate analysis; however in Crohn's disease many factors are interrelated. Therefore, in an effort to understand better the influence of the most important variables on recurrence, we used multivariate regression analysis to identify independent predictive factors and assess their impact on the risk of developing a recurrence requiring operation.

A second issue in analyzing the outcome of patients with Crohn's disease is to characterize the location of the gastrointestinal tract that is involved. Because the macroscopic involvement by Crohn's disease can be widespread, often it cannot be classified simply into the time-honored subdivision of jejunoileal, ileocolonic, and colonic disease. As a result we classified the patients according to location and then identified those with multiple-site *versus* single-site disease to characterize more accurately the widespread nature of the disease activity.

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Address reprint requests to Fabrizio Michelassi, M.D., University of Chicago, Department of Surgery, 5841 S. Maryland Ave., Chicago, IL 60637.

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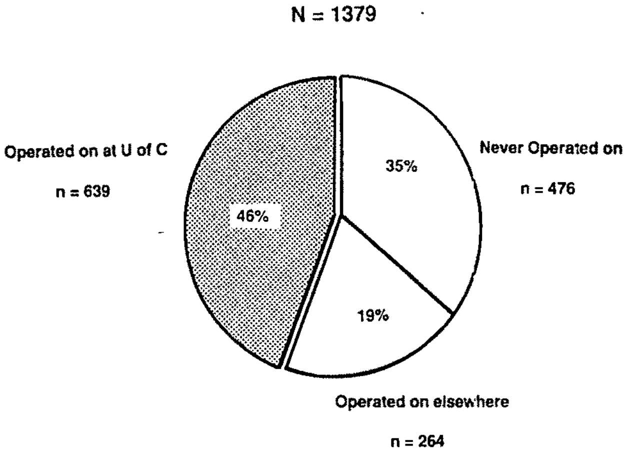

FIG. 1. Patients treated for Crohn's disease at the University of Chicago between 1970 and 1988. Seven hundred forty patients did not undergo any surgical procedure although 264 of them had already undergone operation at least once for complication of Crohn's disease before referral. The remaining 639 patients required at least one operative procedure.

Materials and Methods

Between January 1, 1970 and December 31, 1988, 1379 patients (700 men and 679 women) were treated for Crohn's disease at the University of Chicago Medical Center (Fig. 1). Seven hundred forty patients did not undergo any surgical procedure, although 264 of them had already undergone operation at least once for complications of Crohn's disease before being referred to us. The remaining 639 patients required at least one operative procedure and constitute the data base for this retrospective analysis.

The clinical records of these patients were reviewed and in 605 patients (95%) complete follow-up was obtained. Patients were contacted directly with a questionnaire if not seen at the University of Chicago Medical Center between July 1988 and June 1989. Data on age (at diagnosis of disease and at operation), sex, location of disease, type of operation, intraoperative findings (fistula, abscess, perforation, mass, obstruction), length of resected bowel, persistence of unresected gross disease, histopathologic characteristics of resection margins, morbidity, and death were specifically sought in each instance. Location of disease along the gastrointestinal tract was defined as single site if macroscopic Crohn's disease was confined to one of the following segments or multiple-site if more than one of the following segments was involved: gastroduodenum, jejunoileum, terminal ileum, neoterminal ileum, colorectum, or perineum. Recurrence of disease was defined as recurrence or recrudescence of Crohn's disease anywhere along the gastrointestinal tract or perineum requiring an additional operation.

Based on the data from the 391 patients without previous surgery for Crohn's disease, a covariate analysis was performed with Cox regression analysis[1] to determine those variables significantly associated with recurrence. Survival curves were computed using the Kaplan–Meier estimate.[2] Finally in 236 patients with Crohn's disease limited to one gastrointestinal segment and no previous surgical procedure, we analyzed the influence of site on indications for surgery and type of operative procedure.

Results

Six hundred thirty-nine patients (322 men and 317 women) required surgical intervention for their Crohn's disease. Most patients (n = 391, 61%; mean age, 30.5 years) were operated on for primary disease, while the remaining patients (n = 248, mean age 35.9 years) required operation for recurrent disease. The latter group comprised patients with complicated courses requiring reoperation for the second to the fifth time (Table 1). The location of the disease along the gastrointestinal tract is summarized in Table 2. Crohn's disease was limited to one gastrointestinal segment in 368 patients (58%), while the remaining 271 patients had multiple sites involved. Of the 271 patients with multiple-site involvement, 111 underwent operation on more than one intestinal site during the initial procedure.

The need for operation was determined by a combination of factors, among which symptoms and physical and radiographic findings were the most common (Fig. 2). In most instances more than one factor contributed to the decision in favor of surgical treatment. The most common indications for operation (Fig. 3) were failure of medical treatment (n = 215, 33%), presence of a fistula (n = 154, 24%), and partial or complete bowel obstruction (n = 141, 22%).

Table 3 lists the most frequent intraoperative Crohn's disease-related complications: a fistula (Fig. 4) was the

TABLE 1. *Previous Surgical Treatment*

Procedure	Small Bowel Resection	Ileocolectomy	Colectomy	Perineal Fistulotomy	Miscellaneous
First	53	77	35	64	19
Second	18	16	14	32	5
Third	6	7	5	15	1
Fourth	3	3	1	8	—
Fifth	—	1	1	5	—

TABLE 2. *Location of Crohn's Disease*

Location	Number
A. Limited to only one gastrointestinal segment (n = 368)	
Gastroduodenal	3
Jejunoileal	46
Terminal ileum	203
Neoterminal ileum	56
Colon and rectum	45
Perineum	16
B. Multiple gastrointestinal segments involved (n = 271)	

most common, followed by abdominal abscess (Fig. 5), abdominal mass (Fig. 6), ureteral obstruction (Fig. 7), and complete small bowel obstruction. In 582 patients (92%), a resection was necessary with primary anastomosis in 416 (65%), a temporary stoma in 124 (20%), and a permanent stoma in 42 patients (7%). The remaining 57 patients underwent nonresectional procedures including stricturoplasty, bypass, and drainage of abscesses (Table 4).

Two patients died during the postoperative period, yielding a hospital mortality rate of 0.3% (Table 5). Despite preoperative malnutrition and high doses of exogenous steroids, the morbidity rate was limited. Septic complications, such as wound infection (3.8%), abdominal abscess (1.6%), and intestinal obstruction (2.8%) were the most common postoperative complications.

We evaluated the rate of Crohn's disease recurrence requiring additional operation for the 639 patients who underwent operation. Follow-up data were obtained in 95% of cases. One hundred eighteen patients (18%) developed a recurrence requiring reoperation. Recurrence rates were 20% at 5 years and 34% at 10 years. The recurrence involved the previous anastomosis in 58 patients and it was confined to the afferent limb in 42 cases and to the efferent limb in 16. In nine patients the recurrence occurred proximal to the previous anastomosis, and in nine cases it occurred distal to it. In 35 cases the recurrence was in the perineum and in three instances at the site of a sigmoid resection performed during the repair of an ileosigmoid fistula. In four cases recurrence occurred on a permanent ileostomy.

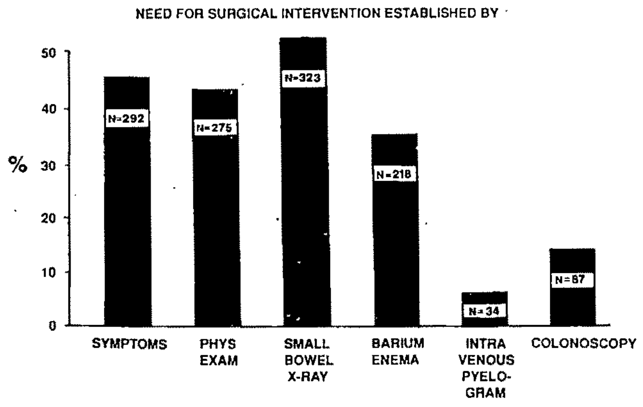

FIG. 2. Symptoms, physical, and radiographic findings were the most common factors determining the need for surgical intervention in 639 patients operated on. In most instances more than one factor contributed to the decision to perform surgery.

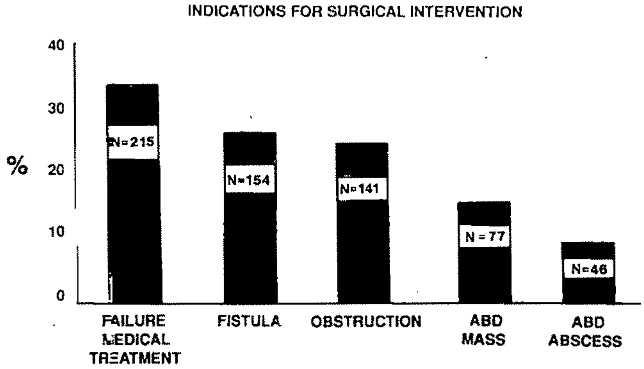

FIG. 3. Failure of medical treatment, the presence of a fistula, and partial or complete bowel obstruction were the most common indications for surgical intervention in 639 patients operated on.

To investigate those factors that might correlate with recurrence, we analyzed the outcome for the 391 patients on whom we operated for primary Crohn's disease. A covariate analysis, incorporating demographic data, findings at operation, surgical procedures, and histopathologic characteristics was performed to determine those variables significantly associated with recurrence requiring reoperation.

The results of this analysis revealed that multiple-site *versus* single-site involvement was the only factor significantly associated with an increased intra-abdominal recurrence rate ($p < 0.001$). The relative risk of intra-abdominal recurrence for patients with multiple-segment disease was about 2.5 times (95% confidence interval: 1.46 to 4.53, using the Cox regression model) that of patients with single-site involvement. Furthermore the relative risk

TABLE 3. *Intraoperative Crohn's Disease-related Complications*

Complication	Number
Patients with at least one fistula	222 (34.7%)
Entero- or cologastric fistula	14*
Enteroenteric fistula	51*
Enterocolorectal fistula	132*
Enterocutaneous fistula	46*
Colosigmoid fistula	5*
Entero- or rectovaginal fistula	26*
Entero- or colovesical fistula	36*
Abdominal abscess	110 (17.2%)
Abdominal mass	55 (8.6%)
Complete small bowel obstruction	23 (3.6%)
Ureteral obstruction	37 (7.7%)

* Some patients had more than one fistula.

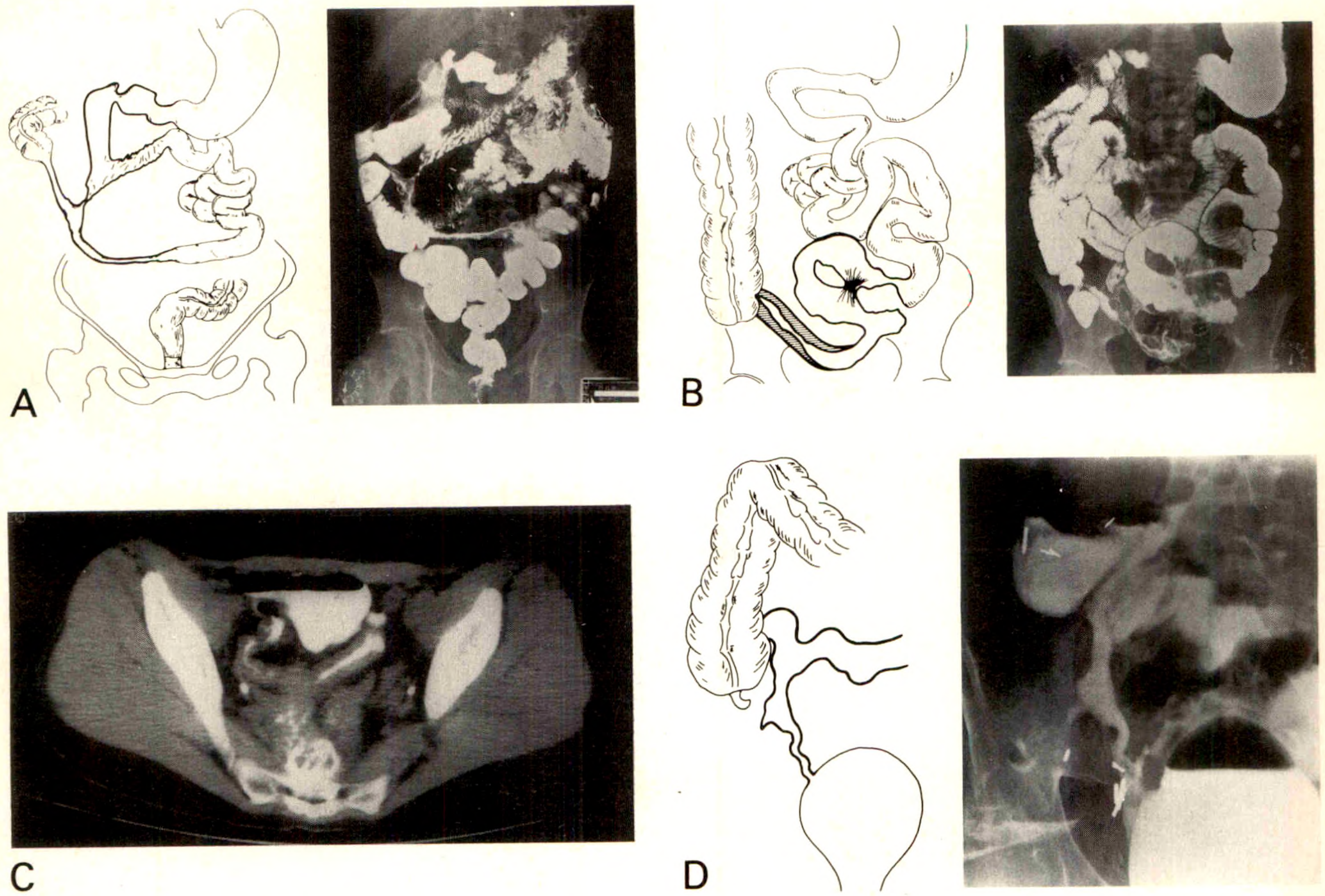

FIG. 4. A fistula was the most common intraoperative Crohn's Disease-related complication. (A) Upper gastrointestinal series showing an ileoduodenal fistula in a patient with a recurrence proximal to the ileoascending anastomosis. (B) Small bowel followthrough series showing an enteroenteric fistula and Crohn's disease of the terminal ileum. (C) Computerized axial tomography showing a thickened, diseased terminal ileum with extravasation of contrast in the perirectal space. Contrast is also seen in the rectal lumen and at exploration an ileorectal fistula was confirmed. (D) Cystogram revealing an ileovesicle fistula.

associated with single-site or multiple-site disease was approximately constant with time during the 15-year time period studied. Thus the annualized risk of recurrence was about 4% for patients with multiple-site involvement and 1.6% for patients with single-site involvement (Fig. 8).

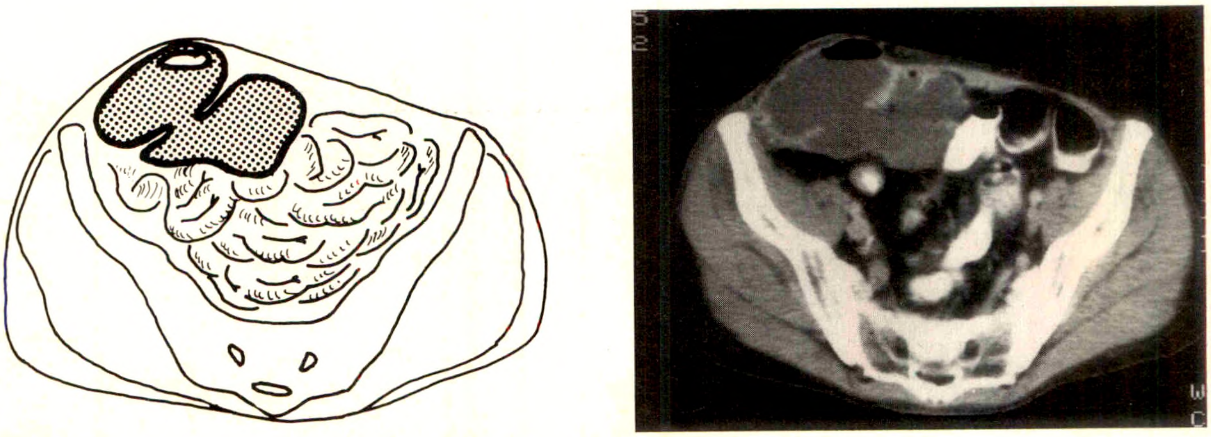

FIG. 5. Computed axial tomography showing a right lower quadrant abscess originated from Crohn's disease of the terminal ileum and perforated through the abdominal wall into the subcute. An air–fluid level is evident.

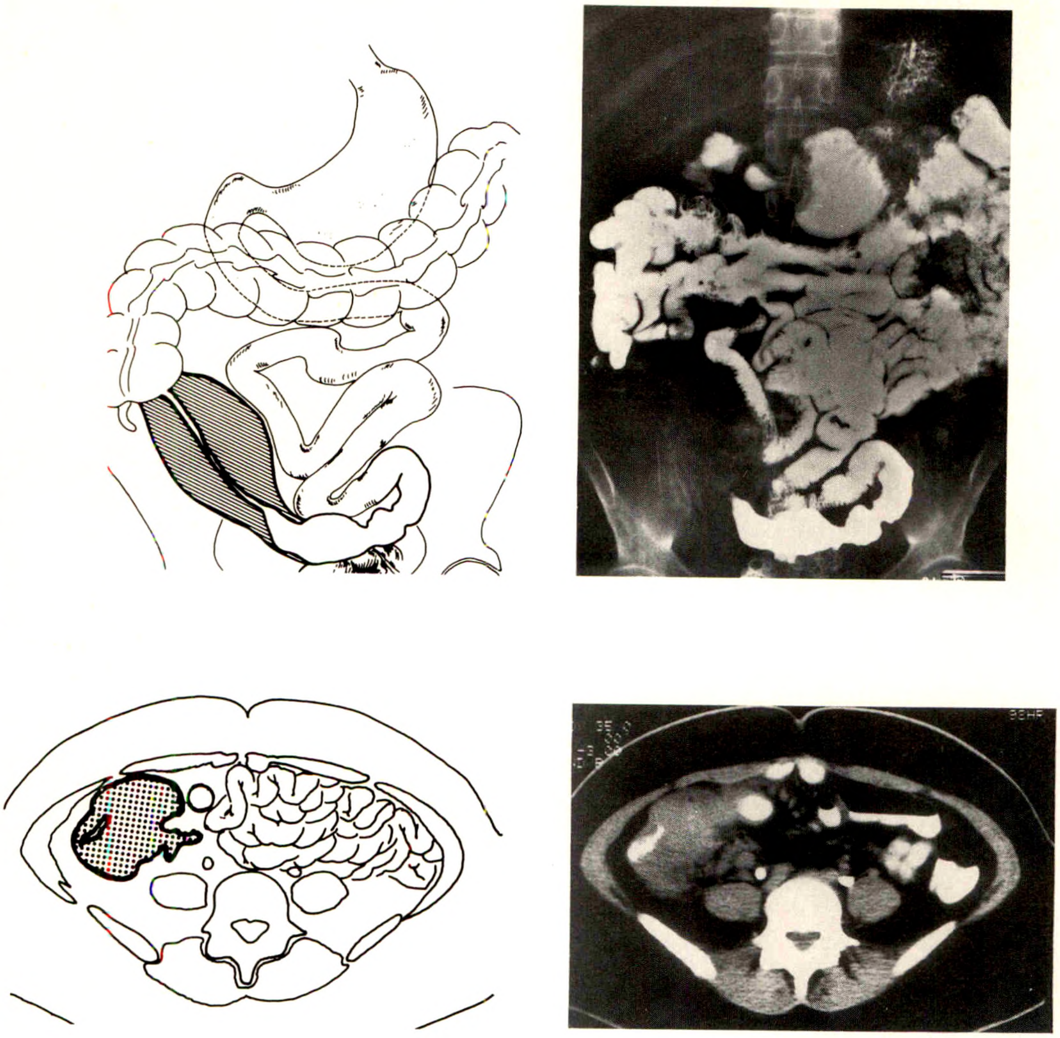

FIG. 6. Upper gastrointestinal series and computed axial tomography showing a large right lower quadrant mass in a patient with disease confined to the terminal ileum. Note the diminished lumen size at the level of the terminal ileum and the displacement of several loops of small bowel from the right lower quadrant.

When perineal recurrence was also considered, the presence of perineal disease was associated with a significantly higher risk of local recurrence ($p < 0.02$). The relative risk of local recurrence for patients with perineal disease was about three times (95% confidence interval: 1.54 to 6.38) that for similar patients who did not have perineal disease.

Gender, age at diagnosis of disease and at time of operation, type of surgical procedure and of re-established gastrointestinal continuity, operative technique, length of resected bowel, and intraoperative findings such as fistula, abscess, mass, perforation, obstruction, and stenosis were not significantly associated with increased recurrence rate. Both persistence of unresected gross disease and a composite variable that included information about site in the gastrointestinal tract and single-site *versus* multiple-site location were significantly associated with an increased rate of disease recurrence in need of reoperation on a preliminary univariate analysis; however when those variables were included in the multivariate analysis they were no longer significant.

Subanalysis of the 236 patients with single-site involvement but no previous operation allowed us to study the influence of location of disease on indications for surgery and type of operation performed. Nine patients had either a gastroduodenal or a perineal location of their disease, leaving 227 patients with jejunoileal, ileocolonic, or colonic localization. The characteristics of jejunoileal and terminal ileal disease were similar. For both sites the common indications for operation were failure of medical treatment, intestinal obstruction, fistula, and abdominal mass (Fig. 9). In contrast one half of the patients with single-site colonic disease were operated on because of failure of medical treatment. Site involved also influenced the procedure performed in that primary anastomosis was feasible in 88% of jejunoileal and terminal ileal cases and a temporary ileostomy was necessary in only 12%. No patients with small bowel location required a permanent

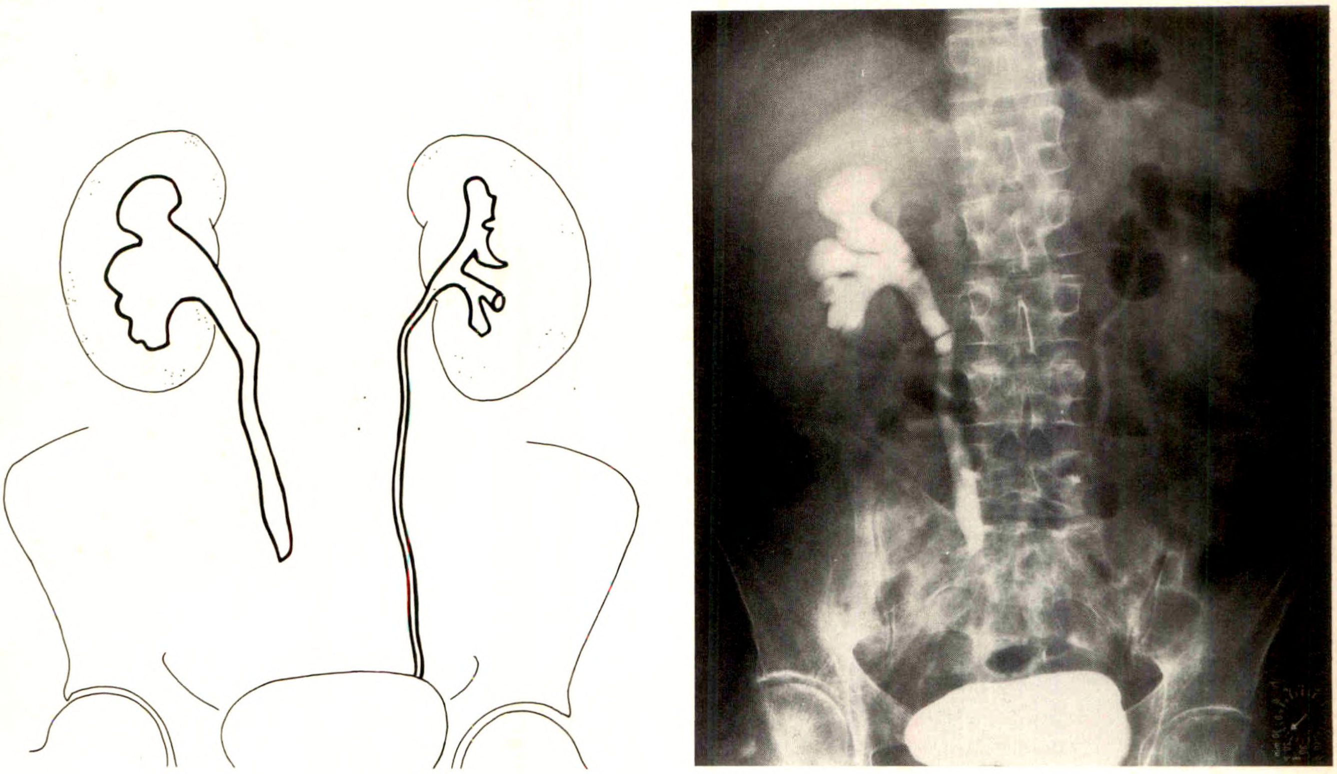

FIG. 7. Complete right ureteral obstruction with ipsilateral hydronephrosis.

stoma. A resection with primary anastomosis was feasible in only 32% of patients with colonic disease. The remaining two thirds of patients required either a temporary or a permanent stoma (Fig. 10), a fivefold higher risk of stoma placement than small bowel disease.

Discussion

For more than two decades there has been an effort to identify those Crohn's disease patients at high risk for recurrent disease requiring reoperation.[3-10] This effort has been complicated by the use of different criteria and definitions.[11] Because we were trying to evaluate a recurrence rate that could be applied broadly to a Crohn's disease population, we included disease considered to be either recrudescent (*i.e.*, occurring in already diseased bowel), or recurrent (*i.e.*, occurring in bowel, where all macroscopic disease had been removed) that required additional operations.

Applying this definition to all patients followed at our institution, we found a recurrence rate of 20% for the first 5 years and 34% for the first 10 years after operation. When limiting our evaluation to those patients on whom we performed the initial operation for Crohn's disease, we found a similar recurrence rate of 20% for the first 5

TABLE 4. *Surgical Procedures*

Procedure	Number of Patients
Resection with primary anastomosis	416
Resection with temporary stoma	124
Resection with permanent stoma	42
Stricturoplasty	5
Bypass	8
I&D perirectal abscess	29
Anal fistulotomy	10
Stoma	3
Drainage of abdominal abscess	2

TABLE 5. *Postoperative Death and Morbidity*

	n	%
Postoperative death	2	0.3
Wound infection	24	3.8
Wound dehiscence	3	0.5
Abdominal abscess	10	1.6
Intestinal obstruction	18	2.8
Anastomotic dehiscence	1	0.2*
Enterocutaneous fistula	3	0.5
Stomal abscess	7	4.2†
Stomal necrosis	1	0.6†
Stomal retraction	1	0.6†

* Calculated on 417 patients with primary anastomosis.
† Calculated on 166 patients with temporary or permanent stoma.

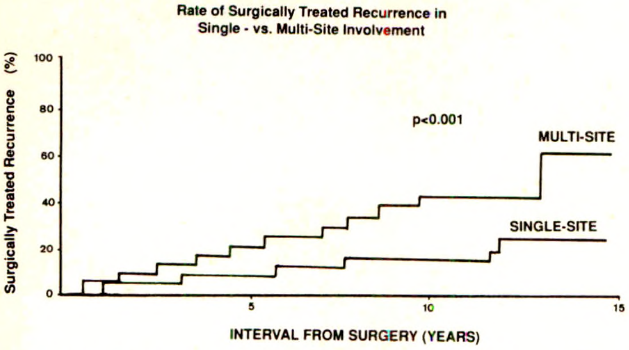

FIG. 8. Rate of intra-abdominal recurrence in need of operation in patients with Crohn's disease originally involving a single or multiple gastrointestinal sites. Patients with disease involving multiple gastrointestinal sites had a relative risk of intra-abdominal recurrence in need of surgical treatment, which was about 2.5 times that of patients with single site involvement ($p < 0.001$). The relative risk associated with single- or multiple-site disease was approximately constant over time. Thus the annualized risk of recurrence was about 4% for patients with multiple site involvement and 1.6% for patients with single-site involvement.

years and 31% for the first 10 years. Our results are consistent with those of Lock et al.,[12] who, using the need for a second resection as the definition for recurrent disease, found a recurrence rate of 31% at 8 years among 337 patients who underwent "curative" resections for Crohn's disease between 1955 and 1973.

To identify possible risk factors associated with higher recurrence rates, we evaluated only those patients who had their first operation for Crohn's disease at our institution. In this way we could accurately assess time to disease recurrence requiring reoperation, be certain as to the observations considered for analysis, and select a homogeneous group of patients without previous surgery for complications of Crohn's disease.

We also used a multivariate analysis to identify those variables that were not interrelated and that would be independently associated with higher risk of recurrence. Univariate analysis yielded three variables that were significantly related to recurrence, but two of them seemed to reflect the same information. The variable representing the surgeon's opinion that macroscopic disease was not resected at the time of surgery was significantly associated with a higher recurrence rate. However when that variable was included in the multivariate analysis it was no longer significant. The surgical opinion seemed to be related to the presence of multiple-site activity, which was the more powerful predictor of disease recurrence. A fourth variable that was significant in the univariate analysis was a composite variable that included information about site location and multiple-site activity. In the multivariate analysis, this variable was also less predictive of recurrence than multiple-site activity alone.

By using a multivariate regression analysis, we found that of all the variables evaluated, only the presence of multiple-site disease and perianal disease significantly predicted a higher recurrence rate. When only intra-abdominal recurrence was considered, only the presence of multiple-site disease was a significant factor. When perineal recurrence was considered, only the presence of perineal disease was also associated with a significantly higher risk of local recurrence. Perineal disease did not increase the risk of intra-abdominal recurrence rate, independently

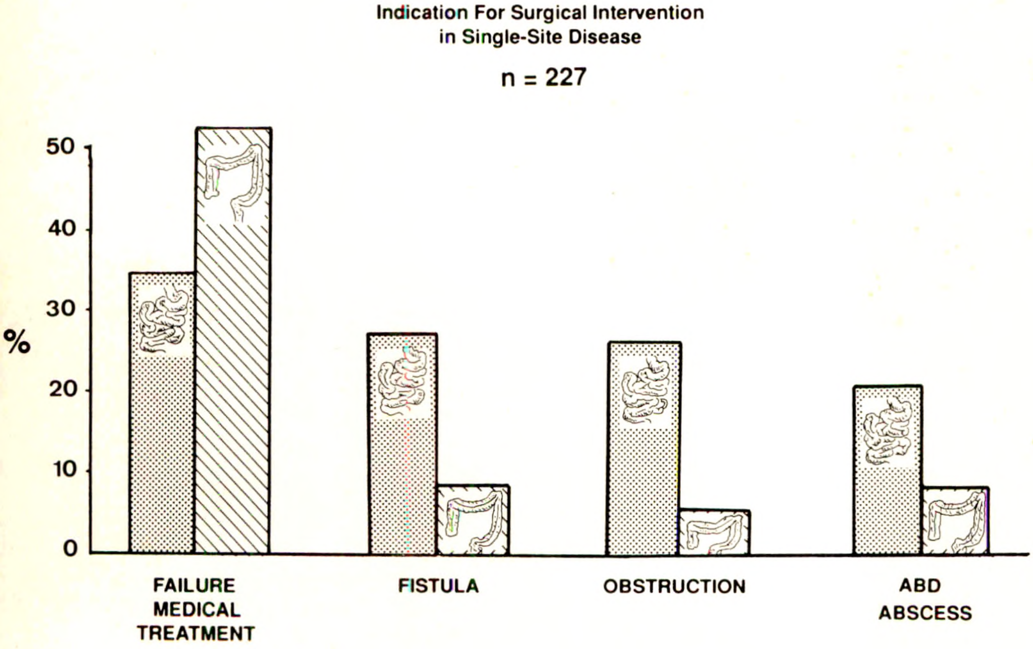

FIG. 9. Failure of medical treatment, intestinal obstruction, and presence of a fistula or an abdominal mass were common indications for surgical intervention in small bowel disease (jejunal-ileal and terminal-ileal localization). In contrast, in single-site colonic disease, failure of medical treatment represented the indication for surgical intervention in more than one half the patients.

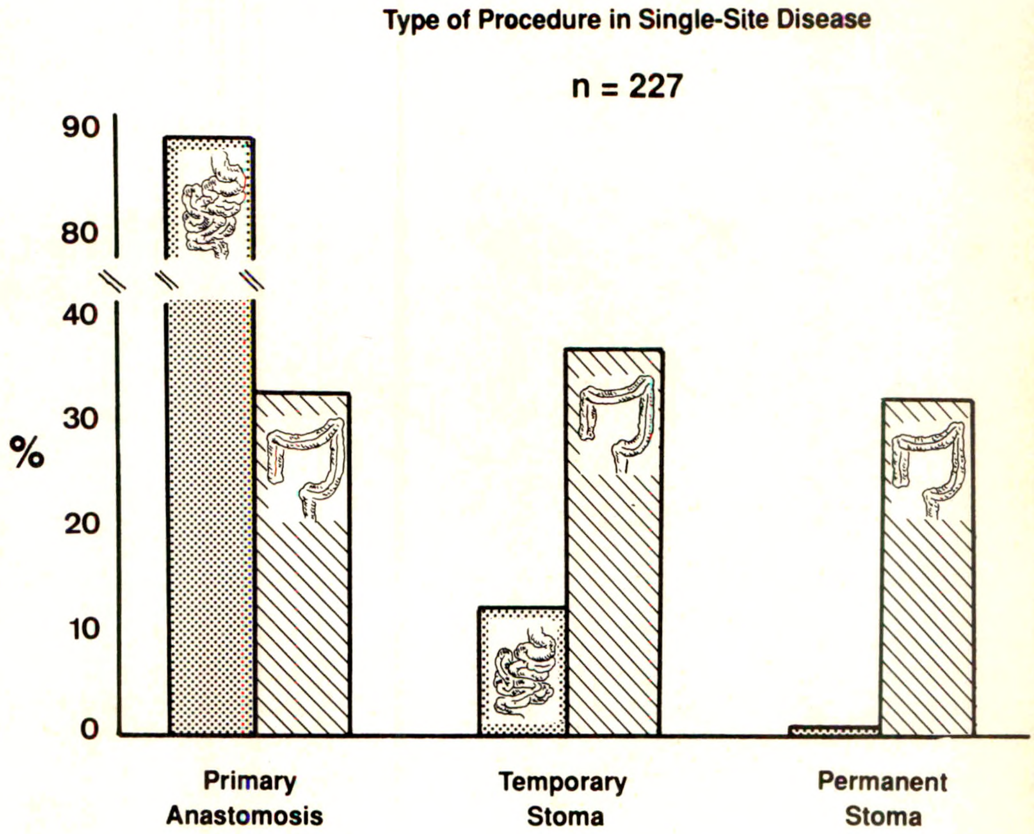

FIG. 10. A resection and primary anastomosis was feasible in most patients with small bowel disease (jejunal-ileal and terminal-ileal localization) and a temporary ileostomy was necessary in only 12%. No patients with small bowel localization required a permanent stoma. A resection with primary anastomosis was only feasible in one third of patients with colonic disease. In the remaining two thirds, the patient required either a temporary or permanent stoma.

of possibly being part of multiple-site disease. In contrast to other reports, we did not find that sex,[13] age at the first resection,[14] duration of the disease before primary resection,[15–17] or presence of microscopic disease at margins of resections[18] was significantly associated with increased recurrence.

It is important to note that we defined patients with multiple-site disease (n = 271, 42%) as those who underwent operation on different intestinal sites at the same time for Crohn's disease (n = 111) or who had disease in different segments, although the operative intervention was only on one of these segments (n = 160). We used this classification because the more traditional approach, which identifies only the segment most actively involved with Crohn's disease, fails to characterize accurately the widespread nature of the disease activity in patients with multiple-site involvement.

The largest group of patients with multiple-site disease was represented by those with ileocolonic disease. In common with other authors, we used the definition of ileocolonic localization for patients with disease in both the small and large bowel. Patients with disease activity limited to the large bowel were classified as colonic and patients with localization of the disease only in the terminal ileum were classified as terminal ileal. The distribution of Crohn's disease along the gastrointestinal tract in our patient population was similar to that in other studies that followed the same definitions for classification of location. The largest group of patients with single-site disease is represented by patients with Crohn's disease of the terminal ileum, followed by patients with colonic and jejunoileal involvement.

Our findings are in agreement with previous reports that the localization of Crohn's disease carries predictive value. For example Whelan et al.,[19] using a definition for recurrence of disease as reoperation for reappearance of Crohn's after an original definitive operation, reported that patients with ileocolonic disease had the highest recurrence rate (53%) compared with colonic (45%) and small intestinal (44%) disease during a follow-up period of more than 10 years (not statistically significantly different). Farmer[20] showed a 27% recurrence rate following resection of active Crohn's disease in both the terminal ileum and large bowel, compared to 21% and 16% for small bowel and colonic involvement, respectively. Data from the National Cooperative Crohn's Disease study analyzed by Mekhjian[21] also showed that the hazard for reoperation for Crohn's disease was higher in the ileocolonic group. Finally a review[16] of 13 recent studies showed that the average surgical recurrence rate was 39% for ileocolonic disease, 34% for large bowel disease, and 29% for small bowel disease.

It is not surprising that multiple-site disease should be associated with higher recurrence rates. Recent research suggests that there is microscopic involvement throughout the gastrointestinal tract with Crohn's disease.[22-25] McLeod[26] has speculated that patients with more "aggressive" disease may have more widespread microscopic disease. Our findings that multiple-site disease was about 2.5 times more likely to be associated with recurrence than single-site disease and that this risk remained constant during a period of 10 years support this concept.

We also found that disease location influenced both the indications for and the type of operation performed. Colonic disease was associated with a higher incidence of operation for failure of medical treatment and with a fivefold greater need for stoma placement than other sites. Permanent stomas were required only in patients with colonic disease.

We conclude that location of disease has value in predicting clinical outcome: the presence of multiple sites of disease involvement and the presence of perianal disease are each associated with a significantly higher risk of developing intra-abdominal or perineal disease recurrence, respectively. With multiple-site disease, the risk for intra-abdominal recurrence is 2.5 times that of single-site disease. With perianal disease the risk of local recurrence is tripled. Finally the presence of colon involvement significantly increases the requirement for a stoma.

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DISCUSSION

DR. JOHN CAMERON (Baltimore, Maryland): It seems as if 25 years ago, in any institution that was caring for patients with Crohn's disease, there were always one or two patients dying of postoperative complications with multiple enterocutaneous fistulas. They have virtually disappeared from all of our wards. I am not exactly sure why; perhaps it is better nutritional support. From our data, that appears to play only a minor role. Perhaps it is better timing of surgical intervention.

Their mortality rate of two deaths among 639 patients is characteristic of what the mortality rate for operating on this disease is today, and the morbidity rate has decreased likewise.

I was somewhat surprised to see that the second most common indication for surgical intervention, however, was the presence of a fistula. Without question, if the fistula is into the bladder or into the vagina, or perhaps a cutaneous fistula, that is an indication for surgical intervention.

Our data, however, suggest that if it is an entero-entero or enterocolonic fistula, and the patient is asymptomatic, the natural history is such that many of them subsequently on barium studies cannot even be demonstrated, and that patients can go for long periods without requiring surgical intervention.

I would like to ask the authors whether of not fistulas in the absence of significant activity of disease or symptoms are continued to be used as an indication for surgical intervention.

Their recurrence rates, at 5 years and at 10 years, of 20% and 34% seem low, and I wonder if they estimated their recurrence rates on the basis of colonoscopy, barium studies, and other means, or if they just used the need for reoperation as indications of a recurrence?

Finally in recent years there has been a good deal of attention paid to the presence or absence of disease at the resection margin, and what that means in terms of disease recurrence. Data from Scandinavia and more recently the Mayo Clinic suggest that if you leave microscopic or macroscopic disease behind at the resection margin, recurrence rates are much higher.

Our data from Baltimore suggest that you can leave microscopic disease behind at the margin, even at times macroscopic disease, without an increased clinical or operative recurrence rate. And I wonder whether they have relooked at their margins and have any further data to supply to this controversy.

Concerning their observations of an increased recurrence rate when there is perineal disease present, I wonder what their explanation is of that. Did perineal disease correlate with multiple site involvement and therefore was not an independent predictor? Did perineal disease give an indication of activity of the disease, and if perineal disease was present, does that mean the disease was more active, or is it just a marker of recurrence?

They made many fine points in the presentation, and I would like to compliment the authors for their contributions.

DR. JOSEF FISCHER (Cincinnati, Ohio): This is an excellent series that really has been very well presented, and as Dr. Cameron has already commented, there is an extraordinarily low mortality rate, and the recurrence rate is substantially lower than most other series that have been followed for 15 years. The fact that these are recurrences requiring surgery really does not change that, because most series that list recurrence do in fact deal with recurrence as requiring surgery, although many of the papers, to read them, are not clear on that point.

There are four points that I would like to address and I have questions for the authors. The first is the question of whether or not the persistence of disease in multiple sites is really counted as a recurrence, or is it counted as a persistence? In other words, if you have ileal disease and gastroduodenal disease, that's a multiple site of disease. Now presumably in such a patient, not all the sites were resected, and if that is the case, if the disease comes back, have you only counted that as recurrence if it recurred in the small bowel, for example, but not if it recurred in its activity in the gastroduodenal area? So are we dealing with persistence as far as the two and a half times incidence of recurrence, or are we dealing with true recurrence?

The second point is the nature of the resection. Now this is an 18-year series, and presumably the intraoperative management of Crohn's disease changed over that period, and I would probably guesstimate that what has happened over the 18 years—and correct me if I am wrong—is that the resections have tended to become more conservative in time, so that perhaps 18 years ago there was a rather larger margin taken rather than a smaller margin now.

As I interpret the manuscript, which the authors were kind enough to give me, most of these resections, at least lately, have been what we would call skimped resections. And if that is the case, I assume, because you did not mention it in the manuscript, that there was no major recurrence or major lesion at the site of the anastomosis at the time of surgery. Is that correct? And therefore are you only dealing with microscopic disease when it finally came back on the permanent section?

The third area Dr. Cameron has already mentioned, and that is whether or not an intrabowel fistula, an ileocolonic fistula, or ileal fistula really constitutes an indication for operation. I would tend to agree with Dr. Cameron that, at least in my own practice, it is not our practice to operate on asymptomatic patients.

Dr. Michelassi did mention an ileoduodenal fistula as an indication, however. Those tend to be symptomatic, and we would agree that that is an indication for operation.

And the fourth question is, I was intrigued by your statistics that approximately a quarter of the recurrences are really in the efferent limb of the anastomosis, that, as I recall, of the 62 patients, in 46 of them the recurrence was on the proximal side of the anastomosis and the remainder were on the distal side. This of course flies in the face of the conventional wisdom that Crohn's disease is a panenteric disease that is stimulated by something from the environment that is swallowed, and that it accumulates in the valvelike area proximal to the anastomosis. Is that a real finding, and if that is a real finding, how did you really diagnose that from proximal disease?

DR. J. THOMPSON (Galveston, Texas): I had an opportunity, as a recent visitor, to look at these data, and I am sure all of you realize they are excellent. Whatever Dr. Michelassi and George Block are doing, it is achieving a better result than almost anybody else. John Cameron can say they are similar to ones he is familiar with, but they are certainly not similar to the ones that I am familiar with, and I think these are really exceptional.

There are a few things I do not understand about this paper. And one of them is, I would like the authors to briefly comment on the fate of stricturoplasty, because people go around talking about that like it is a real operation, and I have really been afraid to do that in a live person. They write about it with some enthusiasm, so I would just like to know what is the fate of these stricturoplasties and do they do them on really long strictures or just on little bitty things that they might have ignored otherwise?

But the thing that I am fascinated by and that others have commented on earlier is these perineal fistulas. I have never really understood the role of perineal fistulas in Crohn's disease, and I wondered if they had studied them. Do they all come from the colon? Do some of them come from the small bowel? Which percentage comes from which? And do they have different life histories? Do those that come from the colon tend to recur more often? Are they more virulent than those that come from the small bowel? Are they handled differently?

You mentioned that it is a bad sign, and I certainly would agree with our experience that it is a bad sign.

You also mentioned that you are sometimes vexed with the *recurrent* fistulas in the perineum, and I would like to ask you, what do you do with those? Do you have someone you like to send them to? I would like his address!

DR. FABRIZIO MICHELASSI (Closing discussion): I would like to thank Dr. Cameron, Dr. Fischer, and Dr. Thompson for their comments and questions. Both Dr. Cameron and Dr. Fischer have asked question about the indications for operating on a fistula. We do agree with them that enterovaginal, enterovesicle, enterocutaneous, and enteroduodenal fistulae are to be operated on. In terms of enterocolonic or entero-enteric fistulae, we operate only on those that either are symptomatic or are associated with findings on physical examination, or cause malabsorption because of shunt and bypass of a major portion of the intestine.

Concerning the issue of the microscopic *versus* macroscopical gross disease: Over the years at the University of Chicago the policy has been to remove the macroscopic gross disease without pursuing the microscopic margins with extension. At surgery it is often imperative to open the specimen, to even look in the afferent or efferent, to see whether there are other macroscopic diseases such as small ulcers that may have been missed on examination of the bowel from the outside. But there is no search for microscopically clean margins. And indeed, when we entered the data available in the multivaried analysis—and there were a handful of patients where the permanent section showed that granulomas were in the resection margin—there was no statistical significance.

Regarding the recurrence rate, I would like to define this as the need for reoperation. There are several ways to define recurrence, depending on how actively and with what means recurrence is investigated. There is endoscopic recurrence, which is quite frequent in the first 12 to 18 months after surgery. There is symptomatic recurrence, and also there is radiologic recurrence.

But clearly, the end point that everybody can be sure of is a recurrence that was in need of being reoperated on where histopathologic findings were correlated with radiologic findings, endoscopic findings, and the patient's symptoms.

There is the issue of persistence of disease. In some patients, disease is left behind because patients are not symptomatic, and in the effort to save as much small bowel or large bowel as possible, conservative resection is done. If there is recurrence in the segment of previously diseased bowel, we have characterized this as a recrudescence in our paper. If one looks at recrudescence *versus* recurrence (meaning disease that was not there at the time of prior surgery), and performs a univariant analysis, the presence of gross disease at the time of surgery left behind and not resected is statistically important.

But then if you enter the gross disease left behind at surgery in the multivariant analysis, the presence of single-site *versus* multi-site involvement is much more powerful from a statistical point of view, and the significance of gross disease left behind is lost.

We found 25% of recurrences at second surgery being localized in the efferent limb of the anastomosis. As you remember, I mentioned that 55% of all patients have a recurrence that occurs at the anastomosis on the proximal side, and probably about 10% of them will have a recurrence that occurs on the distal side. There are about 20% or 30% more patients that do have recurrences away from the anastomosis. I do not have an explanation why in some cases, in most of the cases, the recurrence is macroscopically proximal to the anastomosis and in some cases it is distal.

We looked at different ways to do anastomosis, for example, end to end or end to side, or one layer *versus* multiple layers. None of those variations had any significance on the recurrence rate and on the location of the recurrence.

There was not too much experience with stricturoplasty in the period 1970 to 1988. A little more experience has been accumulated since. So I cannot give you any specific answer, Dr. Thompson, regarding how the stricturoplasties were done. Certainly if a skip lesion is very localized and away from major disease, that can be handled with a stricturoplasty rather than with a second operation. Our intent is usually to limit the number of stricturoplasties, and in contiguous major disease all lesions are handled together.

The management of a perineal fistula or perineal disease is very frustrating both for the patient and for the surgeon and the physician. These patients, once they develop perineal disease, have a three times higher risk than the normal population of having new fistulae recur. Although it is easy to incise and drain an abscess and maybe do a fistula repair also, in the long run these patients run the risk of having functional incontinence.

The reason is not really clear why perineal disease is so frequent in these patients and in a higher proportion than normal patients, although probably 80% to 90% of these fistulae are originating from the anorectal region and specifically from the valve of Morgagni. Whether there is an impaction that occurs more in these valves is really not clear. Why these patients with Crohn's disease are more prone to develop fistulae from impaction of fecal material is also not clear.

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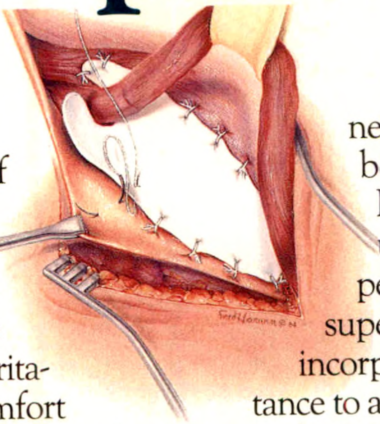

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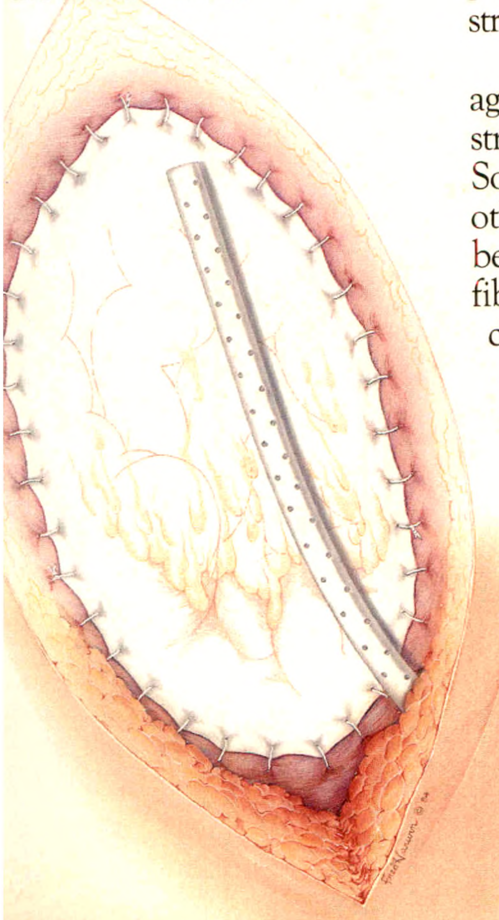

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INDICATIONS AND USAGE

UNASYN is indicated for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions listed below.

Skin and Skin Structure Infections caused by beta-lactamase producing strains of *Staphylococcus aureus, Escherichia coli,* Klebsiella* spp.* (including *K. pneumoniae*), Proteus mirabilis,* Bacteroides fragilis,* Enterobacter* spp.,* and *Acinetobacter calcoaceticus.**

Intra-Abdominal Infections caused by beta-lactamase producing strains of *Escherichia coli, Klebsiella* spp. (including *K. pneumoniae**), *Bacteroides* spp. (including *B. fragilis*), and *Enterobacter* spp.*

Gynecological Infections caused by beta-lactamase producing strains of *Escherichia coli,** and *Bacteroides* spp.* (including *B. fragilis**).

*Efficacy for this organism in this organ system was studied in fewer than 10 infections.

While UNASYN is indicated only for the conditions listed above, infections caused by ampicillin-susceptible organisms are also amenable to treatment with UNASYN due to its ampicillin content. Therefore, mixed infections caused by ampicillin-susceptible organisms and beta-lactamase producing organisms susceptible to UNASYN should not require the addition of another antibiotic.

CONTRAINDICATIONS

The use of UNASYN is contraindicated in individuals with a history of hypersensitivity reactions to any of the penicillins.

WARNINGS

SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (ANAPHYLACTIC) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE APT TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR HYPERSENSITIVITY REACTIONS TO MULTIPLE ALLERGENS. THERE HAVE BEEN REPORTS OF INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY WHO HAVE EXPERIENCED SEVERE REACTIONS WHEN TREATED WITH CEPHALOSPORINS. BEFORE THERAPY WITH A PENICILLIN, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS, AND OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, UNASYN SHOULD BE DISCONTINUED AND THE APPROPRIATE THERAPY INSTITUTED.

SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN, INTRAVENOUS STEROIDS, AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.

PRECAUTIONS

General: A high percentage of patients with mononucleosis who receive ampicillin develop a skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. In patients treated with UNASYN the possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.

Drug Interactions: Probenecid decreases the renal tubular secretion of ampicillin and sulbactam. Concurrent use of probenecid with UNASYN may result in increased and prolonged blood levels of ampicillin and sulbactam. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with UNASYN and allopurinol administered concurrently. UNASYN and aminoglycosides should not be reconstituted together due to the *in vitro* inactivation of aminoglycosides by the ampicillin component of UNASYN.

Drug/Laboratory Test Interactions: Administration of UNASYN will result in high urine concentration of ampicillin. High urine concentrations of ampicillin may result in false positive reactions when testing for the presence of glucose in urine using Clinitest™, Benedict's Solution or Fehling's Solution. It is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix™ or Testape™) be used. Following administration of ampicillin to pregnant women, a transient decrease in plasma concentration of total conjugated estriol, estriolglucuronide, conjugated estrone and estradiol has been noted. This effect may also occur with UNASYN.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been performed to evaluate carcinogenic or mutagenic potential.

Pregnancy

Pregnancy Category B: Reproduction studies have been performed in mice, rats, and rabbits at doses up to ten (10) times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to UNASYN. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed. (See—Drug/Laboratory Test Interactions.)

Labor and Delivery: Studies in guinea pigs have shown that intravenous administration of ampicillin decreased the uterine tone, frequency of contractions, height of contractions, and duration of contractions. However, it is not known whether the use of UNASYN in humans during labor or delivery has immediate or delayed adverse effects on the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obstetrical intervention or resuscitation of the newborn will be necessary.

Nursing Mothers: Low concentrations of ampicillin and sulbactam are excreted in the milk; therefore, caution should be exercised when UNASYN is administered to a nursing woman.

Pediatric Use: The efficacy and safety of UNASYN have not been established in infants and children under the age of 12.

ADVERSE REACTIONS

UNASYN is generally well tolerated. The following adverse reactions have been reported.

Local Adverse Reactions

Pain at IM injection site—16% Pain at IV injection site—3%
Thrombophlebitis—3%

Systemic Adverse Reactions

The most frequently reported adverse reactions were diarrhea in 3% of the patients and rash in less than 2% of the patients.

Additional systemic reactions reported in less than 1% of the patients were: itching, nausea, vomiting, candidiasis, fatigue, malaise, headache, chest pain, flatulence, abdominal distension, glossitis, urine retention, dysuria, edema, facial swelling, erythema, chills, tightness in throat, substernal pain, epistaxis and mucosal bleeding.

Adverse Laboratory Changes

Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:

Hepatic: Increased AST (SGOT), ALT (SGPT), alkaline phosphatase, and LDH.
Hematologic: Decreased hemoglobin, hematocrit, RBC, WBC, neutrophils, lymphocytes, platelets and increased lymphocytes, monocytes, basophils, eosinophils, and platelets.
Blood Chemistry: Decreased serum albumin and total proteins.
Renal: Increased BUN and creatinine.
Urinalysis: Presence of RBC's and hyaline casts in urine.

The following adverse reactions have been reported with ampicillin-class antibiotics and can also occur with UNASYN.

Gastrointestinal: Gastritis, stomatitis, black "hairy" tongue, enterocolitis and pseudomembranous colitis.

Hypersensitivity Reactions: Urticaria, erythema multiforme, and an occasional case of exfoliative dermatitis have been reported. These reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, the drug should be discontinued, unless the opinion of the physician dictates otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with a penicillin (see WARNINGS).

Hematologic: In addition to the adverse laboratory changes listed above for UNASYN, agranulocytosis has been reported during therapy with penicillins. All of these reactions are usually reversible on discontinuation of therapy and are believed to be hypersensitivity phenomena.

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Treatment of Chronic Traumatic Bone Wounds

Microvascular Free Tissue Transfer: A 13-Year Experience in 96 Patients

JAMES W. MAY, JR., M.D.,* JESSE B. JUPITER, M.D.,† G. GREGORY GALLICO, III, M.D.,*
DOUGLAS M. ROTHKOPF, M.D.,* and PAULA ZINGARELLI*

*From the Division of Plastic and Reconstructive Surgery,
Departments of Surgery* and Orthopedic Surgery,†
Massachusetts General Hospital, Harvard Medical School,
Boston, Massachusetts*

During a 13-year period at the Massachusetts General Hospital, Boston, Massachusetts, 97 microvascular free tissue transfers have been performed for soft-tissue reconstruction in 96 patients following bone debridement for chronic traumatic bone wounds. These 96 patients comprise a continuation study of 18 original patients reported in 1982. During a 13-year follow-up period (mean, 77.1 months), 95.8% of these 96 patients have enjoyed complete wound closure with a lack of drainage after the debridement and free tissue transfer. Most of the patients (89.6%) encountered in this study are ambulatory without assist and 5.2% of patients have undergone amputation. Twenty-three per cent of patients required subsequent segmental bone defect reconstruction in the lower extremity after infection eradication. The pathophysiology of chronic traumatic bony wounds is different from that of chronic hematogenous osteomyelitis and thus a high incidence of long-term successful management can be seen through complete wound debridement and adequate soft-tissue coverage.

DESPITE ADVANCES MADE in medical and surgical management, chronic traumatic bony wounds continue to present a difficult problem in patient management.[1-22] The etiology of bone wounds has shifted from a primary infectious origin to trauma with the increasing mobility of our society.[3,4,23-33]

In 1982, as a preliminary report,[1] we presented 18 patients in whom microvascular free tissue transfers were used to close chronic traumatic lower extremity wounds with no reported recurrence of drainage seen in any patient during a mean 19.3-month follow-up interval.[1] Free tissue transfers[1,34-43] were chosen as the soft-tissue treatment for these patients because the degree of bone debridement would not be limited by the amount of available local reconstructive tissues.[19,20,44-72] The purpose of this report is to present the long-term follow-up data for those initial 18 patients and to add 78 additional patients to this initial group. Thus the total of 96 patients have been followed after bone wound debridement and free tissue transfer reconstruction for a mean 77.1 months.

Patients and Methods

In our series of 96 patients a mean period of chronic bone exposure and drainage was 39.6 months, with a range of 6 weeks to 41 years. The mean age was 32.8 years, with a range from 11 years to 66 years and including 11 female and 85 male patients.

Five criteria for the diagnosis of chronic bone wounds were applied for patient selection in this study,[1] including chronic open draining bony wounds for more than 6 weeks, a positive culture from the wound at the time of debridement,[73,74] histologic evidence of nonliving bone with inflammatory changes, with or without bacteria, and x-ray findings consistent with chronic bone infections. With increasing experience scans[75-102] have now been deleted from our preoperative assessment[103] because intraoperative bone debridement[1,104] is now used in determining with accuracy the extent of bone involvement.[105]

Regional bone wounds included skull (1), upper extremity (1), femur (7), tibia (66), and ankle–foot (21). In all patients a microvascular free tissue transfer operation was carried out, including latissimus dorsi muscle flap with skin graft or skin island (80 flaps) gracilis muscle flap with skin graft (11 flaps), and groin flap (6 flaps). In each case a flap was chosen that adequately provided an abundance of well-perfused wound closing tissues. No

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Presented at the 111th Annual Meeting of the American Surgical Association, April 11–13, 1991, Boca Raton, Florida.

Address reprint requests to James W. May, Jr., M.D., Massachusetts General Hospital, ACC-4, Suite 453, Boston, MA 02114.

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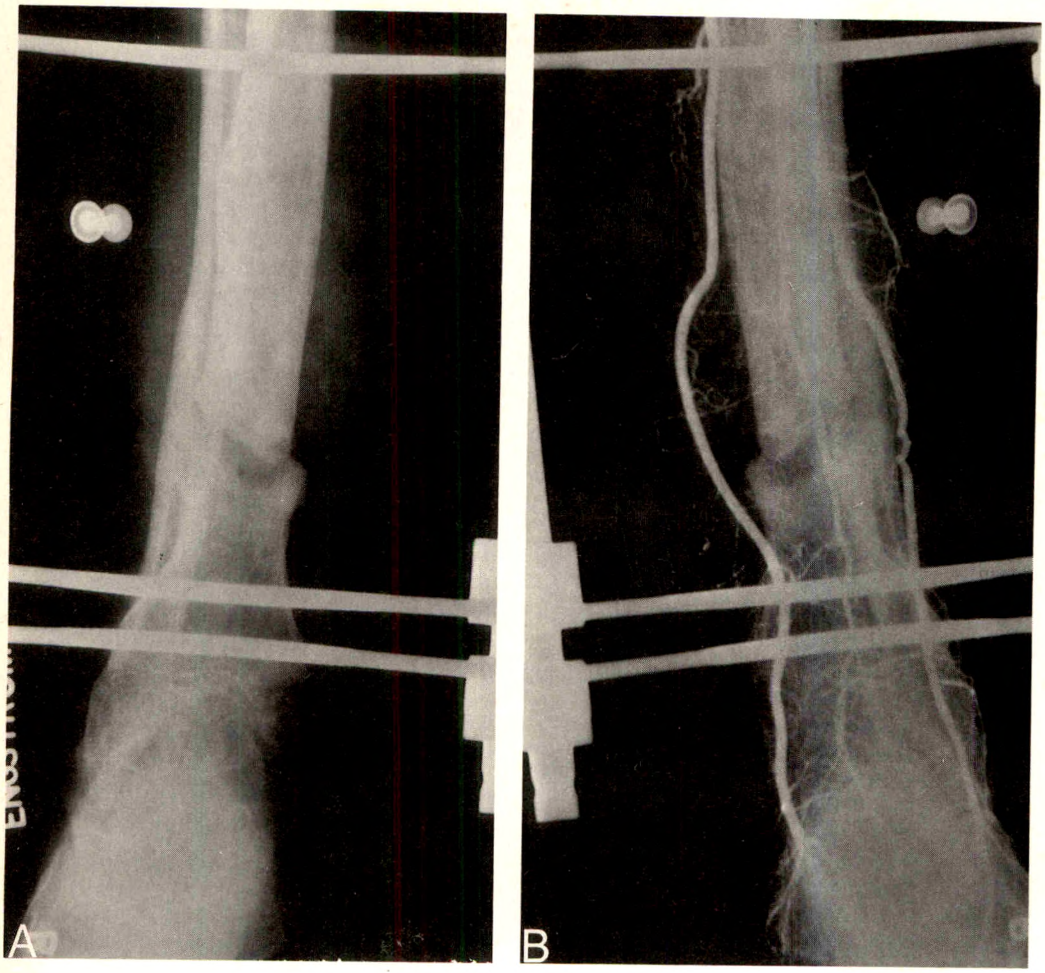

FIGS. 1A and 1B. (A) X-ray of distal tibial nonunion in a 38-year-old woman with a 6-year history of chronic drainage and infected tibial nonunion. (B) Femoral angiogram showing intact posterior tibial artery.

patient was included in whom local healthy flap tissues were available.

Patient Management

Each patient was assessed clinically and a mutual decision was made regarding the possibility of treatment resulting in a functional extremity.[1,105,106] Once decision for debridement and reconstruction was made, an arteriogram[1,105–108] was performed (Figs. 1a and b). An initial wound debridement was carried out under general anesthesia where all exposed and contaminated or necrotic bone was debrided under loupe magnification with a mechanical burr. During debridement cultures and biopsy specimens were taken from bone as well as soft tissue to rule out a chronic cutaneous carcinoma.[44,109,110] The wound was then treated with moist dressings with repeat clinical examination of the wound to be certain that all nonvitalized and devascularized bone had been removed. If gross tissue necrosis persisted, an additional formal debridement was done. At the final procedure, redebridement was performed under intravenous antibiotic administration and a microvascular free tissue transfer (Fig. 2) was performed by two surgical teams working in donor and recipient sites. Free muscle flaps with skin grafts are used almost exclusively, with the procedure lasting between 6 and 8 hours. Immediately before, during, and after the operation, antibiotics were administered intravenously for at least 1 week. Antibiotics were selected by our infectious disease consultants based on culture data achieved at the time of initial bone debridement. In most of our patients, antibiotics were administered intravenously for 6 weeks after operation.[2,111,112] After soft-tissue healing, bone grafting was done if indicated, and 6 months after operatively, as in this case, ambulation and rehabilitation (Figs. 3a to c) were well underway.

Results (Tables 1, 2, and 3)*

*Fate of the Initial 18 Patients**

The original 18 patients have now been followed for a mean of 124.3 months (range, 114 to 156 months). A

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* Study period ended August 1990.

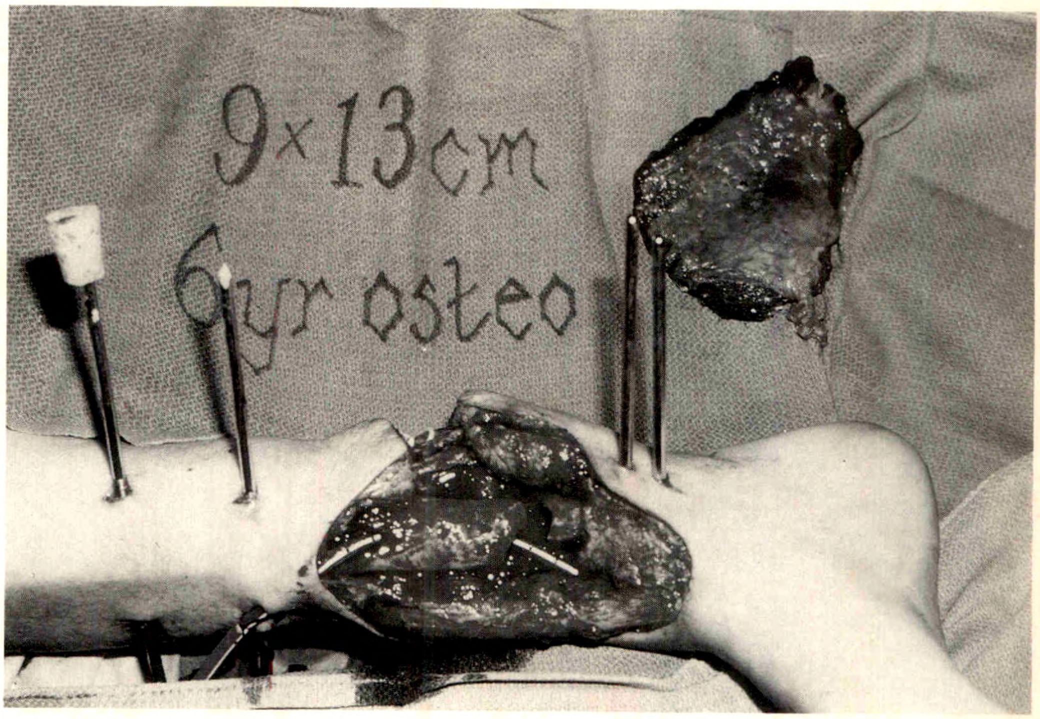


FIG. 2. Bony wound at the time of surgical debridement and free latissimus dorsi flap coverage.

single patient underwent a secondary tibial realignment procedure 1 year after successful wound debridement and closure. Following this procedure the wound drained for 10 days after operation, with antibiotic resolution of culture identified *pseudomonas aeruginosa* organisms similar to those from his original chronic wound. He has united his tibial osteotomy and has remained drainage free for the following 7 years. An additional patient underwent foot amputation for a painful foot and ankle 2 years after successful free flap chronic ankle wound closure. At this time his wound was well healed and vessels were patent by direct operative inspection with negative deep cultures taken at the time of amputation.

Thus recurrence of drainage occurred in 7.1% of the original 18 patients followed for more than 10 years.

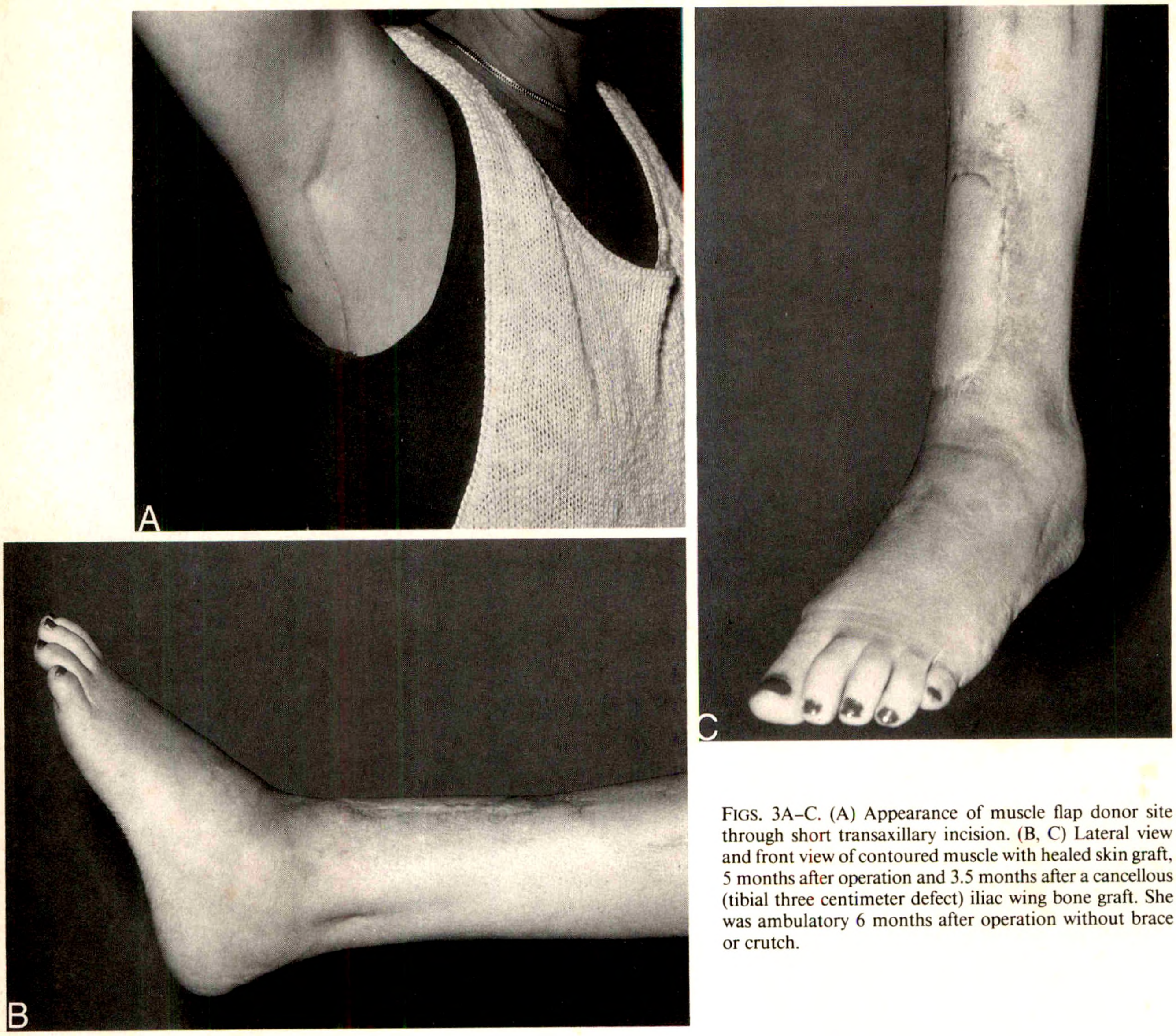
*13-Year Cumulative Study in 96 Patients**

Of the patients in our cumulative study, a single patient had microvascular failure of two sequential gracilis microvascular free tissue transfers. The flaps were debrided in this patient and he ultimately had wound closure by local flap expansion techniques. He has remained drainage free. Thus the ultimate free tissue transfer failure rate in this study was 2.1%.

During the mean postoperative follow-up period of 77.1 months (range, 15 months to 156 months), there have been three additional patients that have manifested further drainage after their initial operative sequence (Table 3). In one patient chronic drainage since 1942 following an attempted knee fusion was unsuccessfully treated by debridement and free latissimus dorsi flap transfer. In this patient drainage reoccurred 24 weeks after the initial flap treatment and has persisted since that time. An additional patient developed drainage from the area of a distal tibial nonunion, which previously had been draining for 6 years. This patient's drainage reoccurred 1.5 years after initial treatment and responded to re-elevation of the flap and secondary removal of the retained sequestrum. After this the patient has remained drainage free for the past 5 years. A third patient underwent a free latissimus dorsi transfer to multiple areas of tibial chronic drainage with recurrent drainage occurring from the distal tibial region within 2 weeks of flap transfer. This area responded favorably to redebridement and adjustment of the initial flap. This patient ultimately underwent an above-the-knee amputation for instability of the knee and poor lower leg function. At the time of the above-the-knee amputation, no evidence of chronic bony drainage or chronic bone infection could be found (Table 3).

Three patients in this series have died (Table 3). The cause of death in this group included metastatic sarcoma, intraoperative anesthetic death at the time of bone graft reconstruction, and nonmedicinal drug overdosage. At the time of patient death, all free flaps were healed without evidence of drainage.

Four patients with wounds of the femur, 3 with wounds of the ankle-foot, and 27 of 66 patients with involvement of the tibia required bone grafting as tertiary procedures. All underwent successful bone graft procedures with



FIGS. 3A–C. (A) Appearance of muscle flap donor site through short transaxillary incision. (B, C) Lateral view and front view of contoured muscle with healed skin graft, 5 months after operation and 3.5 months after a cancellous (tibial three centimeter defect) iliac wing bone graft. She was ambulatory 6 months after operation without brace or crutch.

union, with the exception of two tibial patients who currently have established nonunions (Table 2). The patients with healed bone grafts and all other living patients are ambulatory, with the exception of one additional patient who underwent below-the-knee amputation for a painful bone graft with tibial nonunion, but without evidence of infection (Table 3).

Discussion

The pathogenesis of chronic traumatic bony wounds is substantially different from that of hematogenous osteomyelitis. With hematogenous osteomyelitis,[33] bony infection usually occurs in the sinusoids of the endosteal blood supply[33,113,114] in the metaphyseal region of long bones. Here pathologic bacteria come to rest in a region of relative blood stagnation with low oxygen tension. The infection becomes established in this region and begins to overwhelm the local host response,[115] as pus under pressure begins to spread down the medullary cavity.[33,116] When the advancing infection has overcome the perfusion characteristics of the endosteal blood supply, frank bony necrosis may intervene.[117] This is even more likely in cases when the infection perforates through the bony cortex and begins to elevate the periosteum with additional loss of blood supply. A patient may be treated successfully with intravenous antibiotics alone, but if progressive bone necrosis has occurred, it becomes more difficult for the host defense mechanisms plus antibiotics to counter effectively the persistence of bacteria plus dead bone.[116,117] The ultimate result of this process may be significant bone necrosis potentially extending throughout the length of the involved long bone.[32,33,118]

TABLE 1. *Complications of Free Tissue Transfer Wound Closure Seen in 96 Patients*

Complication	Number	Percentage of total
1. Free flap failure	2	2.1
2. Re-exploration of flaps	7	7.3
3. Recurrent drainage	4	4.2
4. Brachial plexus neuropraxia	7	7.3
5. Donor/recipient site hematoma	6	6.3
6. Skin graft loss	4	4.2

TABLE 3. *Ultimate Result of Bone Wound Closure and Reconstruction in 96 Patients**

Result	Patient Number	Percentage
Drainage recurrence	4	4.2
Amputation for inadequate leg or foot	5	5.2
Died	3	3.1
Tibial nonunion	2	2.1
Ambulatory without assist	86	89.6

* Percentages total more than 100% because some patients are included in more than one category.

This well-established pathophysiologic process should be contrasted with a *dissimilar* process seen in most cases of traumatic bone infection. Under traumatic circumstances a perfectly healthy and viable bone becomes invaded by bacteria through traumatic introduction. During this process bony[119-125] and soft-tissue blood supply in the region of trauma may be interrupted, creating severe tissue damage and cellular death[126,127] in varying degrees to the bone and soft tissues.[128] Under these circumstances, if the wound were sterile and could be closed with well-perfused soft tissues, the devascularized bone could be invaded by vascular cutting cones and ultimately would be revascularized and repopulated with osteocytes.[117] This is an identical process to that observed to occur when bone grafts are accomplished under sterile conditions or when wounds with minimal contamination are closed with early well-vascularized soft-tissue coverage.[1] However, under traumatic circumstances with a high concentration of inoculated bacteria, an ideal milieu exists devoid of blood supply[129] for the proliferation and feeding of bacteria in the accompaniment of serum-filled dead space. When the bacterial process is active with significant proliferation, the vascular cutting cone mechanism for revascularization is halted.[117] Under these circumstances the extent of necrotic bone is usually limited to that amount of bone initially devascularized because the wound is in fact opened by the traumatic process before it becomes contaminated and infected. Thus pus under pressure cannot extend the bone necrosis process. This is the reverse of what occurs in hematogenous osteomyelitis, which may need to be opened (incised and drained) by the surgeon to avoid the extension of bone necrosis. Thus the extent of nonliving bone seen in traumatic bony wounds is usually dictated by the initial trauma and is not likely to be as extensive as that seen in severe and neglected cases of chronic hematogenous osteomyelitis.[49]

TABLE 2. *Classification of Chronic Traumatic Tibial Bone Wounds (after surgical debridement)*

Classificiation	No. of Patients	Time to Ambulation* (months)
Type I (tibia intact not requiring bone graft)	39	1.5
Type II (tibia intact with bone graft required for functional structural support)	5	5.5
Type III† (tibial defect 6 cm or less, with intact fibula)	14	11.4
Type IV† (tibial defect larger than 6 cm, with intact fibula)	5	12.0
Type V (tibial defect larger than 6 cm, without intact ipsilateral fibula)	3	12.7

* Time to ambulation without crutch or upper extremity assist.

† A single patient underwent amputation for a painful leg in this group.

If the differences in the pathophysiology as described can be translated into practice management, then it should be possible in traumatic chronic bony wounds to debride completely the localized ischemic[100,130-134] or nonliving bone and soft tissues and to close the wound with well-perfused tissues.[49,135,136]

We have selected a subset group of our patients who have required free tissue transfers as our study group to test this hypothesis. In this group the size of the flap chosen to fill the defect was not limited by the availability of local tissues. Thus the bone and soft-tissue debridement could be based only on disease removal considerations rather than the concern always present when local tissues are used as to whether there will be enough tissue to close the wound.[1,49]

In this 13-year study, 4 of 95 patients (4.2%) with successful free transfers have manifested recurrent drainage after initial treatment. After secondary treatment only a single patient remains with recalcitrant drainage. An additional subgroup of patients (five) underwent amputation. This group comprises one patient who had persistent drainage and an unsatisfactory extremity and four patients in whom, in retrospect, an incorrect judgment was made as to the salvageability of the patients' lower legs and feet. It is our feeling that the initial drainage recurrence rate in four patients represented our inability to recognize and debride completely the wound and should not suggest that the thesis of long-term, successful wound treatment in chronic traumatic bony wounds is not possible. It is of interest to note that of the three patients with tibial chronic bony wounds who failed to maintain wound closure, all three had type I (intact tibia) tibial status after wound debridement. In two of these patients it was recognized that a borderline debridement was done to avoid making the tibia unstable, thus avoiding a more complex defect bone graft reconstruction. Thus ironically it was psychologically easier for the surgeon to debride a tibia that already had a defect than it was to debride an intact tibia to the point that a defect was created to solve the bone infection problem.

Experimentally it has been shown that well-perfused muscle flaps[108,137] with increased oxygen[7,130,138–143] deal with bony wounds in an optimal way and promote wound healing.[144] In the experience of others[4,11,29,35,42,43,145] and in the discussion of our initial patient presentation,[1] it was noted by experienced workers in this field that one should not overstate the findings of our preliminary report.[146,147] We fully agreed with this position and stated it in our initial report: "one cannot say that chronic bone infection was permanently cured or prevented in any patient in this study." However we went on to state that it was encouraging that the mean drainage-free period of 19.3 months for the patients in our initial report was seen[1] considering that most patients with failure of treatment of chronic bony wounds will recur within the first year after treatment.[4,148] If one looks at the long-term results of our original 18 patients presented in 1982,[1] there has been recurrence of drainage in a single patient with antibiotic resolution during a mean follow-up period of 116 months (10.3 years). This 7.1% drainage recurrence rate is in the same range as our 4.2% drainage recurrence rate seen in our overall cumulative series followed for a mean 77 months. It is of interest that in the overall series our four recurrent drainage patients drained within a mean 7 months of the their original treatment, making it unlikely that the overall group recurrence rate will be significantly greater than that of our initial 18 patients.

The primary cause, in our series, of failure in chronic bony wound treatment was inadequate bone debridement.[1,149] The microsurgical free tissue transfer has done more in our patient management to allow freedom of debridement than any other single development in the management of patients with chronic bony wounds. If only a small amount of nonliving bone and significant bacteria are left behind,[150] an established sequestrum and seed for ultimate failure over time may ensue.[1,42,105,135,150]

It is possible that the degree and the extent of bone infection could require a debridement, which is inconsistent with skeletal reconstruction from a practical view point. Under these circumstances draining wound maintenance or even amputation surgery may be in the patient's best interest.[151,152] Using our recently developed classification[105] for chronic bony wounds of the tibia (Table 2), our ability has been enhanced in advising the patient as to the time and bone reconstructive commitment that will be needed to achieve a functional limb.

We continue to be enthusiastic over the use of free muscle transfers[1,105,153,154] covered with skin grafts.[105,155,156] This method of wound closure provides minimal donor site deformity, and with denervated muscle[157–161] used to fill the recipient site, excellent wound contour has been achieved and maintained[162] during the length of our study. Of course a free tissue transfer should only be used when an adequate and well-tolerated local means of soft-tissue wound closure is not available.[133,105] However the decision as to how much bone and soft tissue to debride should be based on local tissue viability and not on the volume of local reconstructive tissue available to close the wound.

It would appear that chronic traumatic bony wounds are theoretically successfully treatable by thorough debridement and soft-tissue reconstruction. One would find little disagreement with the concept that extremity amputation can cure chronic bony wounds.[151] Thus the concept of local wound (bone and soft tissue) amputation with extremity salvage by soft- and hard-tissue reconstruction should not seem illogical. A 4.2% recurrence rate during a mean 77-month follow-up period in these 96 patients compares favorably with other reports[5,9,11,29,30,35,43,145,152,163–165] and methods.[4,5,7,8,11,133,166–188]

The treatment of chronic traumatic bony wounds is difficult and it is not surprising that significant pessimism has existed concerning the cure of such patients.[35,147,189] On the other hand, it is possible that this pessimism[31] in part has been based on the lack of total involved bone and soft-tissue wound debridement in the face of inability to completely cover the recipient wound with well-vascularized tissues. Perhaps some day "cure rates"[4] will be reported in patient series with chronic traumatic bony wounds rather than disease-free interval.[4,190] We believe that the freedom of debridement provided by the almost-unlimited microsurgical free tissue wound closure technique has provided a very high chance of successful long-term wound management in patients with chronic traumatic bony wounds. It would appear that the results from our 13-year study are consistent with this interpretation. Bony reconstruction of the lower extremity after infection eradication is the major challenge facing reconstructive surgeons in the future.

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DISCUSSION

DR. M. J. JURKIEWICZ (Atlanta, Georgia): Dr. May had 190 references in this article. Clearly he researched the literature. He neglected a significant paper, however, that was published in 1920 by Alan Kanavel in *Surgery, Gynecology, and Obstetrics.* It was read before the Chicago Surgical Society on the management of wounds with noncollapsing walls. Kanavel observed that dead space management in patients with such wounds was enhanced by flap transposition, and he usually used muscle.

He applied the principle with success in patients with osteomyelitis of the femur and of the mandible, with chronic empyema, and with chronic draining sinus tracts of the frontal bone, pelvis, and lower abdomen.

These data by Dr. May and his colleagues confirm the observations of Kanavel, unaccountably lost and forgotten until the past decade.

So too do our data at Emory, compiled by George Cierny of the Orthopedic Service and Foad Nahai on the Plastic Surgery service. There were 189 patients treated in this 5-year period, 91 by transposition of muscle and 98 by free flap transfers, similar to the data from the MGH. There was a 97% long-term relapse-free rate in this 5-year period. There were no free flap losses at all in this series.

Our patients are evaluated for treatment options by taking into account not only the extent of trauma, nonviable dead bone, and wound factors all under the rubric of dead space management, but also by considering host factors.

May I ask what factors you take into account in an attempt to answer the often crucial question of amputation *versus* attempt at limb salvage?

You rely on 6 weeks of IV systemic antibiotics based on recommendations of your infection disease consultants. If our patients are candidates for 6 weeks of antibiotics, Cierny incorporates this into the dead space management by appropriate antibiotic-impregnated beads. These beads then are removed in 6 weeks, preserving the cavity and thus facilitating the secondary bone grafting. About half of our patients are so treated and the other half by primary grafting as part of the initial dead space management. I wonder if you would comment on this approach.

Finally I think you need to elaborate on the 7.3% rate of neuropraxia in harvesting the donor muscle in your series.

I believe the study has broad implications in chronic wound management, well beyond osteomyelitis of bone, in treatment of wounds with noncollapsing walls from whatever cause and in whatever location. Consider Kanavel's principle in the use of a well-vascularized flap transfer for wound management.

DR. STEPHEN J. MATHES (San Francisco, California): This paper nicely demonstrates the versatility of microvascular composite tissue transplantation for coverage of complex wounds, whether they be located in the lower extremity or in other areas.

Of interest in 1983 here in Boca Raton, I had the opportunity to present a paper entitled "Coverage of the Infected Wound," which also included a review of the use of muscle flaps in a retrospective group of patients who presented with complex open infected wounds. My study included 27 patients with chronic osteomyelitis involving the lower extremity. Because all these patients are now well beyond 5 years since their surgery, it is of interest to report to you their current status. Since their debridement and flap coverage, three of these patients did have recurrence of infection. Two of these went on to have a second muscle flap and are also now 5 years beyond the second flap without recurrence of infection. Thus of the total 27 patients, 26 are free of osteomyelitis, resulting in a 96% success rate after a minimum of 5 years' follow-up. The one patient who did have recurrence refused further treatment and underwent a below knee (BK) amputation. These results, like those shown today, demonstrate a treatment that can provide cure for a complex wound.

I have four questions I would like to ask Dr. May. The first relates to his definition for the entry point for patients into a study. I am concerned about the short duration of the open wound of 6 weeks. In most of our studies in the surgical literature, we usually define a chronic wound as in the range of 6 months.

Along the same line, one might wonder how many of these patients truly had osteomyelitis entering into the medullary space, or were some of these superficial cortical infections?

The second question relates to debridements. How many debridements were performed and what was the timing of the muscle flap? Some of us have tried to perform the debridement with simultaneous muscle flap closure, and I am interested to hear Dr. May and his group's criteria for timing and also how they determined how much bone debridement is required?

The third question relates to management of the infected nonunion. Some have believed this is a different disease than chronic osteomyelitis, particularly as Dr. Jurkiewicz asked, I wonder has he considered immediate bone grafting with muscle flap coverage? Also what does he see as the role of the Elizera technique, that is, a migration of vascularized bone, without the use of flap coverage?

And my last question relates to antibiotic duration of therapy. I recognize this still represents a controversial area. I know Jim May and I have had many discussions about the length of antibiotics. I have shortened our antibiotic therapy to a 10- to 13-day range.

DR. JOHN R. BORDER (Buffalo, New York): I want to discuss this paper because it once again illustrates the incorrect use of words that plagues every aspect of the field of infection. The essential problems are: (1) We use words that are historical accretions and not defined; (2) Because we use the word we think we understand the pathophysiology; (3) Because we think we understand the pathophysiology we embark on therapy; and (4) The therapy is incorrect, expensive, dangerous, and ineffective.

The title "Chronic Traumatic Bone Wound" is certainly correct. The problem is that it was used interchangeably with "chronic osteomyelitis." There is a condition that presents largely in children that is properly called osteomyelitis. This is a bacterial high-pressure intramedullary abscess. It clearly does everything bad commonly attributed to osteomyelitis and if treated early clearly responds to antibiotics. If the condition is neglected, it clearly creates dead bone that requires removal and may produce major bone loss.

The problem is that this lesion in children has nothing in common with the adult problem except pus. In the adult devitalized bone is created by the original trauma and the by surgical treatment of the trauma. That devitalized bone then provides a protected place for bacterial growth that provides very little nutrient to support bacterial growth. The same trauma also damages and devitalizes the surrounding soft tissue so that it is rapidly converted to avascular scar tissue. These combined events then provide a protected place for bacterial growth, which produces chronic small-volume purulent drainage. The problem here is not the growth of bacteria, as it is in true osteomyelitis, but the wound conditions that support bacterial growth.

Successful early therapy of true osteomyelitis requires vigorous antibacterial measures. Successful therapy of the chronic traumatic bone wound requires revision of the wound so that it does not support bacterial growth. Short-term antibiotics are useful only after this has been accomplished. When the problem is stated this way, it may be seen to have much in common with chronic purulent drainage from a wide variety of prostheses.

The only problems peculiar to bone are that mobility at the fracture site induces purulent drainage even if there is no devitalized bone. Infected fractures if completely immobilized have a major drop in purulent volume and promptly heal if there is no devitalized bone.

The biggest problem in the chronic traumatic bone wound is that the devitalized bone is in continuity with viable bone (fragments are removed early). At surgery it is very difficult to be sure that all devitalized bone has been removed. Practically the wound that continues to drain has retained devitalized bone. A derivative problem has always been that when all devitalized bone has been removed, a major skeletal gap in bony continuity may exist.

All of these problems may now largely be solved granted a correct view of the pathophysiology, emphasizing the wound problem and not the bacteria problem, and properly employing modern technology that allows continued joint function proximal and distal to the lesion. This paper clearly shows how this major improvement in extremity injury therapy has occurred. The keys are clearly the use of external fixators to maintain extremity function while the local lesion is treated, resection of all devitalized bone and avascular scar tissue, the use of a muscle microvascular free flap to provide a well-vascularized wound, cancellous bone grafting for defects of 6 cm or less, and microvascular grafting of the fibula for long defects.

This is a very important evolution. The same principles apply to vascular grafts and artificial joints. The same principles also apply to all wound complications. Our persistence in calling all wound complications wound infections hides the common theme of these injuries and implies that the only problem is bacterial. The problem is always devitalized tissue (in elective surgery, usually hematoma), and many times, devitalized tissue and bacteria.

DR. JAMES W. MAY, JR. (Closing discussion): I would like firstly to thank Dr. Jurkiewicz and Dr. Mathes and Dr. Border for their comments. I was a little concerned about a paper that included 190 references, because I was somewhat worried that an editor of the *Journal* might accept the paper but not the bibliography! I feel somewhat relieved to be able to tell him I can add one more when he asks me to change the number of entities. I would like to thank Dr. Jurkiewicz for reminding us of that important work of Dr. Kanavel.

Dr. Jurkiewicz has asked what factors affect our judgment as to whether to attempt to eradicate a bone infection or to advise the patient to have an amputation. I tried to address this firstly in my slide showing the characterization of tibia chronic osteomyelitis or chronic bone infection, because the patient should have a pivotal role in knowing what is ahead of him or her in the ensuing months.

In essence, as Dr. Jurkiewicz implied, the problem is not the eradication of the infected milieu: the problem in most cases is the reconstruction of a useful extremity. In a single operation, therefore, we can almost always achieve an eradicated bacterial milieu, but the problem then rests in reconstructing the bony anatomy of the lower extremity.

There are other secondary findings that influence the decision, such as metabolic disease and patient age. One could make a list almost infinitely long. After lengthy consultation with the patient, the surgeon begins to get a feel for the willingness of the patient to make a commitment. This is really what it is all about. Saving the leg and completing reconstruction takes much longer than amputation.

Intravenous antibiotics are a very important and exciting area that impact chronic bone wound management. Dr. Jurkiewicz has mentioned the use of beads, (antibiotic-impregnated methylmethacrylate) that can be left in the bed of the wound, with a muscle flap placed on top after complete bony debridement. The beads maintain a space beneath the flap and are later replaced by a bone graft.

We have used this only on uncommon occasions, because usually a perfused muscle can be so nicely fitted into the confines of the debrided leg.

One should remember why beads are used at all, however. In the orthopedic literature over a decade ago, methylmethacrylate was first used to hold hip replacements into place. This was among the first substances to be mixed with antibiotics that allowed a time-related release of antibiotic material. Perhaps we should not be narrow in our thinking about the use of methylmethacrylate as an antibiotic release mechanism. Perhaps we should develop antibiotic release devices that are not related to the cement capability of methylmethacrylate at all.

The methylmethacrylate bead system also has a major drawback in that antibiotic peak doses occur at about 48 to 72 hours. With all of the currently used antibiotics, perhaps advances could be made in a staged release system that would be much more effective than our current methylmethacrylate system.

Dr. Jurkiewicz has importantly highlighted the issue of neurapraxia of the brachial plexus during the donor maneuver of the latissimus dorsi transfer. I used the term "neurapraxia" because in none of these patients were symptoms lasting longer than 2 months. It is obviously very devastating, however, when you fix the leg and hurt the arm. We first thought that this might be related to the axial harvesting of the thoracodorsal artery and the subscapular vessels, as they relate to perfusion of the brachial plexus. With time it became clear that arm hyperextension was the problem. Those patients were generated in the mid-1980s, and I am happy to say that this problem is totally rectifiable by proper arm positioning.

Dr. Mathes has raised a number of interesting questions that are important. Firstly the definition of chronic osteomyelitis: If one reads the literature, the definition extends anywhere from 3 weeks to 2 years, depending on the author and the zeal of scrutiny in that definition.

The question really is what does the word "chronic" mean? To some of us it means 15 minutes, and to others it may mean a lifetime.

We have chosen the term 6 weeks, because 6 weeks is a time interval in which most wounds that do not have substantial contamination and dead bone could be expected to be healed. If we remove the patients who had less than 6 months of chronic bony exposure, it deletes about 15 patients from this study. This does not change the results of this work.

Cortical *versus* cancellous bone involvement is an important issue. I tried to outline the severity of bone involvement in our study by the slide showing the percentage of type I tibial bony wounds on through and including type V. The majority of our tibial patients were in type I, involving the cortex of the bone alone, although 41% of the tibial patients in this series had involved bone deep to the cortical bone itself.

Our debridement criteria involved a first-look operation, whereby all bony materials are debrided from the wound in addition to avascular soft-tissues. Usually the definitive debridement and flap closure was done 5 to 7 days later. Some of these patients had two or three debridements, because we were unable to convince ourselves that all the dead bone had been removed. Even under these circumstances, we have demonstrated our inability to accurately debride all dead bone in 4% of our patients.

We would be concerned, I think, about those who would advocate immediate bone grafting because, remember, an immediate nonvascularized bone graft is a dead piece of bone being placed into a contaminated bony wound. We have shown in the laboratory that nothing beats a completely debrided wound, and if you add dead bone plus bacteria to a wound that you have just debrided, then you have added dead bone plus bacteria in a milieu that could lead to persistent drainage or infection. We will be interested to see what Dr. Jurkiewicz and his group, working with Dr. Nahai and Dr. Cierny, have to say about that approach 5 years or so down the line.

The Ilizarov technique is a complicated technique where an osteotomy is performed, for example, in the tibia, and then the tibia is lengthened, making the reconstructive defect in a chronic bone wound management problem much smaller. Certainly we believe that the Ilizarov technique is going to have a major impact on the bony reconstruction of these chronic wounds, but may not replace adequate debridement and soft-tissue reconstruction.

Finally the important question about the length of antibiotic administration brought out by Dr. Mathes deserves a word. A study was done in which the last cohort of the group of these patients in this study were randomized between long- and short-term antibiotics. About 43 patients have been studied, 20 of whom have had only 1 week of antibiotics and 23 of whom have had 6 weeks of antibiotics. There has been a single recurrence of chronic bone wound drainage in each group.

We cannot tell that there is a difference between 1 week *versus* 6 weeks of antibiotics. I would certainly agree with Dr. Border that the issue here is not the infective organism, but rather the cause of the infection.

I also agree with Dr. Border's comments about the classic misuse of terms. We live in the real world, and in that world, words are reflective of the use to which they are put. Therefore when you talk about chronic bone wounds to an infectious disease person, a light does not go on until you call it osteomyelitis. If you talk to a pathologist, they define osteomyelitis as bacteria plus dead bone plus inflammation, and if you talk to a surgeon, we may define it in terms of wound closure and reconstruction.

This confusion in terms, I believe, is one of the reasons that this entity is so poorly understood. I have tried to highlight the differences in pathophysiology between invasive osteomyelitis of hematogenous origin *versus* the commensalism that develops in a trauma patient between bacteria and the bone that has been devascularized by a traumatic insult.

The Role of Prostaglandin E_2 in Immune Suppression Following Injury

JOHN T. GRBIC, D.M.D., JOHN A. MANNICK, M.D., DAVID B. GOUGH, F.R.C.S.I., M.Ch., and MARY L. RODRICK, Ph.D.

From the Department of Surgery, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts

It has been thought for some time that prostaglandin E_2 (PGE_2) released from activated monocytes/macrophages may contribute to the suppression of immunity seen after burns and major injury because PGE_2 inhibits the activation of T lymphocytes. To clarify this issue, we studied 15 patients with total body surface area burns of 20% to 90% (mean, 48%). Peripheral blood mononuclear cells (PBMC) were obtained from these patients one to two times each week for 1 month after burn and were stimulated with the T-cell mitogen phytohemagglutinin (PHA). On 14 occasions the PBMCs from eight patients were significantly suppressed (30% or more) in their response to PHA (suppressed [sup] burn) as compared with PBMCs from normal controls. In 38 instances PBMCs from 12 patients were not significantly suppressed in PHA (nonsupressed [nonsup] burn). Sup burn PBMCs and control PBMCs were cultured with or without the addition of the cyclooxygenase (CO) inhibitor indomethacin (Indo, 1 μg/mL) and studied for PHA response and the production of the stimulatory cytokine interleukin-2 (IL-2). Indo partially restored the PHA response of sup burn PBMCs to normal. Sup burn PBMCs also were deficient in production of IL-2. Indo increased IL-2 production by sup burn PBMCs significantly more (160% ± 20%, $p < 0.005$) than control (57% ± 5%) and nonsup PBMCs (67% ± 8%). Next inhibition of the PHA response of PBMCs from 12 burn patients and 17 controls was studied by exogenous PGE_2. At all time periods after burn injury, patients' PBMCs were significantly more sensitive to inhibition by PGE_2 (50% inhibition at 10^{-8} mol/L [molar] PGE_2) than PBMCs from normal controls (50% inhibition at 10^{-6} mol/L PGE_2) with maximum sensitivity occurring 8 to 14 days after injury. Peripheral blood mononuclear cells from patients with more than 40% burns were significantly ($p < 0.05$) more sensitive to PGE_2 than those from patients with lesser burns. Interleukin-2 was added to cultures of sup burn PBMC, nonsup burn PBMC, and controls containing 10^{-7} mol/L PGE_2. Interleukin-2 totally reversed PGE_2 inhibition of the PHA response in PBMC from both controls and burn patients. Because endotoxin leak from the gut has been implicated as a trigger for a number of the metabolic and immunologic abnormalities following injury, the authors looked for the effect of a bolus infusion of *Escherichia coli* endotoxin (Endo, 4 ng/kg) in seven normal healthy volunteers on the response of PBMC to PHA and on the production of PGE_2 and IL-2. The PHA response and IL-2 production were both significantly ($p < 0.01$) suppressed 4 hours after Endo, returning to normal by 24 hours. At 4 hours PBMCs were also significantly more sensitive ($p < 0.001$) to exogenous PGE_2. Production of PGE_2 by adherent PBMCs was maintained at normal levels at 4 hours and increased markedly by 24 hours after Endo. The CO inhibitor ibuprofen (800 mg 2 hours before and at the time of Endo infusion) prevented a significant decrease in PHA response and IL-2 production by PBMCs and blunted the increased PGE_2 production 24 hours after Endo. The *in vitro* addition of Indo partially and exogenous IL-2 completely restored the PHA response to normal. Exogenous IL-1 had no effect when added to cultures of PBMCs from Endo-treated volunteers. It is concluded that (1) PGE_2 has a role in the suppression of immunity after burn injury; (2) PGE_2 exerts its suppressive effect principally by inhibition of lymphocyte IL-2 production; (3) endotoxin mimics the effect of injury on PGE_2 production, T-cell activation, and IL-2 production; (4) administration of CO inhibitors is likely to be essential for the success of clinical regimens designed to correct the immune suppression that follows major injury.

DEPRESSION OF CELL-MEDIATED immunity in patients following thermal or traumatic injury has been demonstrated by anergy to recall antigens,[1] delayed allograft rejection,[2] and decreased response to T-cell mitogens.[3] Our laboratory[4,5] has shown that a primary immunologic defect in patients following

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Dr. Grbic's present address is Oral Medicine & Pathology, Columbia University College of Dentistry, New York, NY 10032.

Dr. Gough's present address is University College Hospital, Galway, Ireland.

Address reprint requests to Mary Rodrick, Department of Surgery, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

Address correspondence to John A. Mannick, Department of Surgery, Brigham and Women's Hosptial, 75 Francis St., Boston, MA 02115.

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severe injury was the decreased production of the lymphokine interleukin-2 (IL-2).

The arachidonic acid metabolite, prostaglandin E_2 (PGE_2) has been shown to decrease T-cell activation by inhibiting the production of IL-2.[6] The ability of PGE_2 to inhibit IL-2 production is believed by some investigators to be due to the activation of a suppressor T cell,[7] although this cell, if it exists, has never been well characterized. A more likely explanation of PGE_2 action on T lymphocytes is its well-defined ability to stimulate adenylate cyclase activity and thus to increase intracellular cyclic adenosine monophosphate (cAMP) concentration, a negative signal for T-cell activation. In burn patients, increased levels of PGE_2 have been found in the area of the burn wound[8] and in the adjacent lymphatics[9] and in serum.[8] Antonacci et al.[10] reported an increased conversion of arachidonic acid to PGE_2 by monocytes obtained from burn patients. Wood et al.[11] have shown that the cyclooxygenase inhibitors indomethacin (Indo) and flurbiprofen could increase *in vitro* IL-2 production by splenocytes from thermally injured mice and Faist et al.[12] were able to increase the mitogen responsiveness of circulating lymphocytes from patients with traumatic injury by the addition of Indo.

In this study we examined the role of PGE_2 in the inhibition of IL-2 production in burn patients by determining (1) the effect of the cyclooxygenase inhibitor Indo on the mitogen responses and IL-2 production of peripheral blood mononuclear cells (PBMC) from these patients, (2) whether there was a difference in sensitivity of PBMC to PGE_2 when burn patients were compared with controls, and (3) the effect of the addition of IL-2 to PGE_2-suppressed mitogen responses.

There is a burgeoning consensus that the trigger for many of the immunologic abnormalities noted after traumatic or thermal injury is bacterial endotoxin,[13] either leaked directly from the gut into the portal circulation or produced by intestinal bacteria translocated from the gut in response to injury. Because bacterial endotoxins are well-known stimuli of monocyte and macrophage PGE_2 production, we also studied the effect of the administration of small doses of bacterial endotoxin to normal human volunteers to determine the effect of endotoxin on T-cell mitogen responses and IL-2 production and on the production of PGE_2 by circulating monocytes.

Materials and Methods

Human Subjects

After obtaining informed consent, 15 patients were studied after admission to the Burn Unit of the Brigham & Women's Hospital. Their ages ranged from 18 to 65 years, with a mean age of 35 years. The mean burn size was 48% total body surface area (TBSA), with a range of 20% to 90%. The study was approved by the Committee for the Protection of Human Subjects from Research Risks of the Brigham & Women's Hospital. Venous blood samples were obtained from the patients one to two times weekly until their discharge from the Burn Unit or until their death. Eight of the fifteen patients studied had at least one septic episode as defined by positive blood cultures and/or strong clinical evidence that prompted a course of antibiotics. One of the patients died following the development of sepsis. Thirty normal healthy individuals were used as controls and were age and sex matched whenever possible.

The volunteers for the endotoxin study were 13 normal men (mean age, 33 years; range, 28 to 39 years), who were judged healthy by history, physical examination, hematology, chemical studies, and stress electrocardiogram. They were admitted 4 days before the study to the Clinical Research Center (CRC) of the Brigham & Women's Hospital, Boston, Massachusetts for acclimatization and evaluation. Written informed consent, which was reviewed and approved by the Brigham & Women's Hospital Committee for Protection of Human Subjects from Research Risks, was obtained for all volunteers. The subjects were given a standard hospital diet and activity was limited to the Clinical Research Center. Ambulatory, normal healthy laboratory volunteers were also studied at the same times as infused hospitalized volunteers. Two normal subjects of similar age were studied for each hospitalized volunteer.

Seven volunteers in separate hospitalizations were given either an intravenous bolus infusion of purified *Escherichia coli* endotoxin (4 ng/kg) in saline into an indwelling intravenous catheter or an infusion of saline alone. Venous blood samples were drawn from the volunteers before endotoxin or saline infusion (0 hours) and at 4 and 24 hours after infusion. Six other volunteers received two doses of ibuprofen, 800 mg orally, 2 hours before and at the time of Endo or saline infusion.

Reagents

Roswell Park Memorial Institute (RPMI) 1640 medium, minimum essential medium (Eagle) (MEM), 1-glutamine, n-2 hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), antibiotic-antimycotic solution (5000 units penicillin/5000 μg streptomycin/25 μg Fungizone/mL) (PS), and fetal bovine serum (FBS) were obtained from Grand Island Biological Co. (Grand Island, NY). Beta-2-mercaptoethanol (2-ME) was obtained from Eastman Kodak (Rochester, NY). Fetal bovine serum was heat inactivated at 56 C for 30 minutes and filtered through 0.45-μm Nalgene filters (Nalgene Co., Rochester, NY) before use. Purified phytohemagglutinin (PHA) was a gift of Dr. Peter Polgar. Tritiated thymidine (^{3}HTdr, 6.7 Ci/mmol/L [millimolar]) was obtained from New England

Nuclear (Boston, MA). Purified human interleukin-2 (Lymphocult-TLF) was obtained from Biotest (FRG) (400 units/mL). Recombinant human IL-1 and IL-2 were purchased from Genzyme Corp. (Boston, MA). Indomethacin and PGE_2 were obtained from Sigma Chemical Co. (St. Louis, MO). The Indo and PGE_2 were dissolved in 70% ethanol at concentrations of 2 mg/mL and 1 mg/mL, respectively. These were diluted to their final concentration in complete medium (described below).

Preparation of PBMCs

Peripheral blood mononuclear cells were harvested by centrifugation of heparinized peripheral blood diluted 1:2 in phosphate-buffered saline on Ficoll-Hypaque (Pharmacia, Piscataway, NJ) for 35 minutes at 400*g*. The interface cells were collected and washed three times in MEM with 1% PS, 2 mmol/L glutamine and 1% HEPES (MEM-PS). After the third wash, the cells were placed in MEM-PS containing 5% FBS (complete medium, CM) and counted using trypan blue for viability and Turk's stain for observation of nuclear morphology. Cells were always more than 95% viable. As patients' Ficoll interface cells often have increased contamination with myeloid cells, estimations of mononuclear cells by Turk's stain were made and cell counts adjusted accordingly so the total number of mononuclear cells per well of all cultures was similar. Peripheral blood mononuclear cells were collected from one or more patients or Endo-treated volunteers and two untreated volunteers for each day's tests.

Blastogenesis Assay and the Effect of Indomethacin

Peripheral blood mononuclear cells isolated as above were cultured in 220 μL/well volume at 1×10^5 cells/well in 96-well flat-bottomed tissue culture plates (Nunc, Denmark) containing CM. The T-cell mitogen PHA was added at a concentration of 6 μg/ml for 90 hours at 37 C in 5% CO_2 and the wells were pulsed with 1 μCi ^{3}HTdr during the last 18 hours of culture. Indo (1 μg/mL) was added to some of the wells at the initiation of the assay. The cells were harvested on a multiple automated sample harvester (Cambridge Technology, Cambridge, MA) and the incorporated radioactivity was measured by liquid scintillation counting in an LKB beta counter (LKB Instruments Inc., Gaithersburg, MD). The mean amount of radioactivity was determined from triplicate cultures and expressed in counts per minute. The standard deviation of the mean of triplicates never exceeded 10%. The counts per minute (cpm) of the cultures containing no mitogen (background cpm) were subtracted from the cultures with mitogen and this number was used in all calculations. Patients and normal volunteers were compared and, to avoid interassay variation, suppression of the lymphocyte response was calculated by the formula:

$$\% \text{ Suppression} = 1 - \frac{\text{patient cpm}}{\text{control cpm}} \times 100$$

In addition the percentage enhancement with Indo was calculated for each patient and normal control by the following formula:

$$\% \text{ Enhancement} = \left[\frac{\text{cpm with PHA + Indo}}{\text{cpm with PHA}} - 1\right] \times 100$$

Determination of PBMC Sensitivity to PGE_2

The sensitivity of PBMCs to the inhibitory effects of PGE_2 was determined by the method of Goodwin et al.[15] A total of 1×10^5 PBMCs were placed into culture with 200 μL of CM containing 1 μg/mL of Indo (to prevent *de novo* PGE_2 synthesis), and either 0, 10^{-6}, 10^{-7}, or 10^{-8} mol/L (molar) PGE_2. Twenty microliters of the T-cell mitogen PHA (final concentration: 6 μg/mL) was added, the cultures were incubated, pulsed, and harvested, and incorporated radioactivity determined as mentioned above. The percentage suppression for each concentration of PGE_2 was determined by the formula:

$$\% \text{ Suppression} = 1 - \frac{\text{cpm of cultures with } PGE_2 + \text{Indo}}{\text{cpm of cultures with Indo only (0 M } PGE_2)} \times 100$$

The dose of PGE_2 that caused 50% inhibition (ID_{50}) was calculated by determining the two PGE_2 concentrations more than and less than 50% inhibition and extrapolating from these two points the concentration of PGE_2 that caused 50% inhibition. If the 50% inhibition was more than 1×10^{-6} mol/L or less than 1×10^{-8} mol/L, then the ID_{50} was set at 1×10^{-5} mol/L and 1×10^{-9} mol/L PGE_2, respectively.

Addition of Purified IL-2 to PGE_2 Containing Mitogen Cultures

Peripheral blood mononuclear cells were cultured at a concentration of 1×10^5 cells/well as above with 200 μL of CM containing 1 μg/mL Indo, 10^{-7} mol/L PGE_2, and 6 μg/mL PHA. Twenty microliters of ultrapurified human natural IL-2 (final concentration: 8 units/mL) or the same volume of CM were added to the wells. The cells were incubated, pulsed, harvested, and the incorporated radioactivity determined as above. The percentage suppression compared to Indo alone was calculated as above.

Production of PGE_2 by Adherent Cells

Peripheral blood mononuclear cells (200 μL/well) in CM at a concentration of 5×10^6/mL were incubated in

96-well microliter plates for 1 hour at 37 C. Nonadherent cells were removed by washing with CM. Adherent cells were then cultured in FBS-free RPMI (with antibiotics, HEPES, and glutamine as above) for 24 hours with or without the addition of *E. coli* 055B5 lipopolysaccharide (Sigma Chemical Co., St. Louis, MO) at a concentration of 1.5 μg/mL. Supernantants were harvested and the PGE_2 concentration was measured by commercial radioimmunoassay (Advanced Magnetics, Cambridge, MA).

Production of IL-2

Interleukin-2 was generated by culturing PBMCs at 1×10^5 cells/well in 200 μL volume for 24 hours in CM with or without 2.5 μg PHA/well. Indo was added to some of the cultures at a final concentration of 1 μg/mL. Supernatants were collected and frozen at −70 C until assayed.

IL-2 Assay

Assay of IL-2 was made as previously described.[5] Supernatants to be tested for IL-2 were diluted from 1:2 to 1:64 in medium in 100 μL volume as above and incubated for 1 hour at 37 C, 5% CO_2. CTLL-2 cells were freed of T-cell growth factor (TCGF) by washing three times (800 rpm for 7 minutes) in RPMI 1640 medium containing 1% PS, 1% HEPES, 5% FBS, 2 mmol/L glutamine, and 5×10^{-5} mol/L 2-ME. The cells were then diluted to 4×10^4/mL and 100 μL added to each well. Cultures were incubated for 20 hours at 37 C in 5% CO_2. One μCi 3HTdr/well was added, cultures were harvested 4 hours later, and then counted as above. Units of IL-2 were determined by comparison with a standard TCGF prepared as previously described.[5] The IL-2 content was calculated using a program provided by Dr. Brian Davis (Immunex Corp., Seattle, WA) on a Kaypro 2 microcomputer. T-cell growth factor was assigned a value of 1 unit.

Statistical Analysis

Statistical analyses were performed using a Vax 11/780 computer (Digital Equipment Corp., Waltham, MA) and a standard statistical package (Minitab, University of Pennsylvania, Philadelphia, PA). A normal probability plot was used to detect nonparametric data and the appropriate statistical methods (Student's t test for parametric data and Mann-Whitney U test for nonparametric data) were used.

Results

Enhancement of the Mitogen Response by Indo

To determine whether PGE_2 played a role in suppressing the PHA-induced mitogen responses of burn patients, we studied the effect of the cyclooxygenase inhibitor Indo on the mitogen responses of these patients. The patients' PBMC PHA response data were separated into two groups: (1) those with a mitogen response suppressed 30% or more (n = 14 determinations in 8 patients) compared with controls (sup burn) and (2) those with mitogen responses suppressed less than 30% (n = 38 determinations in 12 patients) (non sup burn). The 30% level was considered to be significant in our laboratory because none of the normal controls were suppressed 30% or more from the mean normal value and this value was calculated to be more than two standard deviations from mean of the normal controls. Our results (Fig. 1) indicate that PBMCs from normal controls (n = 65 determinations) and the nonsup burn patient cultures were enhanced 12% ± 3% and 13% ± 3% (mean ± standard error of the mean [SEM]) by Indo, respectively. The mitogen responses of the sup burn PBMCs were enhanced significantly more, 43% ± 5% (mean ± SEM) ($p < 0.001$), by the addition of Indo when compared to normal controls and nonsup burn PBMCs. However the responses of the sup burn PBMCs were not returned to the level of the normal controls by Indo. The mean suppression observed in the sup burn group was 49% and after the addition of Indo, the suppression decreased to a mean of 28%.

Enhancement of IL-2 Production by Indomethacin

Because PGE_2 has been shown to function by inhibiting the production of IL-2,[6] we examined the effect of Indo

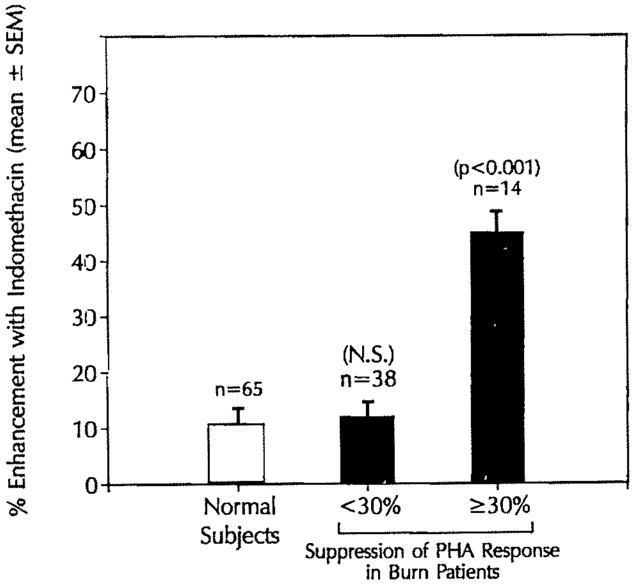

FIG. 1. Ability of indomethacin to enhance the PHA response of burn patients' PBMCs. Indomethacin significantly enhanced ($p < 0.001$) the PHA-induced blastogenesis of sup burn PBMC (see text) when compared to PBMCs from both normal controls and the nonsup burn groups (n = the number of determinations in each group). NS, not significant.

on the IL-2 production of burn patients' PBMCs. The cell cultures were separated again into two groups: (1) cells from patients with an IL-2 production that was suppressed 50% or more compared to the mean of the normal controls (sup burn) (n = 28 determinations in 10 patients) and (2) cells from patients with an IL-2 production that was less than 50% suppressed (nonsup burn) (n = 39 determinations in 14 patients). The 50% suppression level was considered significant in our laboratory because none of normal controls was found to have his/her IL-2 production suppressed more than 50% and this value was approximately two standard deviations from the mean of the normal controls. The patients were compared to 15 normal controls (mean age, 32 years) whose mean 24-hour IL-2 production was 1.46 ± 0.11 units (mean ± SEM). The results (Fig. 2) demonstrate that PBMC from all three groups exhibited increased IL-2 production with the addition of Indo. However the sup burn group was significantly more enhanced (160% ± 20%) (mean ± SEM) ($p < 0.0005$) than both the normal control (57% ± 5%) and nonsup burn (67% ± 8%) groups. The IL-2 production of the sup burn group was 0.37 units, which was enhanced to 0.96 units with the addition of Indo; however this remained lower than the level of normal controls (1.46 units).

It has been reported that PGE_2 may affect the IL-2 assay by suppressing the proliferation of the IL-2–dependent cell line directly,[14] although this finding has been disputed by other investigators.[7,8] We tested the proliferation of the CTLL-2 cells by adding concentrations of PGE_2 normally found in cultures of PBMCs (10^{-7} mol/L to 10^{-8} mol/L) with the standard preparation of TCGF in several assays and found no significant effect of PGE_2 on these cells directly (10^{-7} mol/L = 0.92 units; 10^{-8} mol/L = 0.93 units; 0 mol/L = 1.00 units). In addition Indo had no direct effect on the proliferation of the CTLL-2 cell line. Therefore these findings suggest that a metabolite of the cyclooxygenase pathway, presumably PGE_2, inhibited IL-2 production by PBMCs of burn patients.

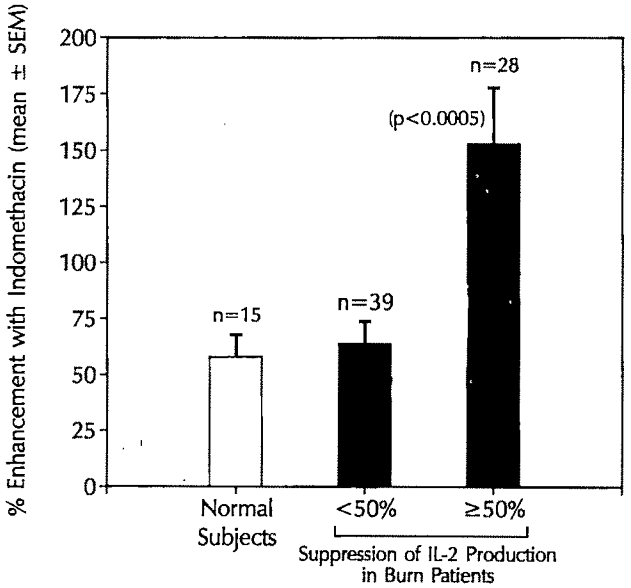

FIG. 2. Enhancement of IL-2 production by indomethacin in burn patients. Burn patients' PBMCs were divided into a nonsuppressed group (see text) and a suppressed group and compared with normal controls. In all three groups, IL-2 production by PBMC was enhanced by indomethacin, although the sup burn group was significantly ($p < 0.0005$) more enhanced than either the normal controls or the nonsup group (n = the number of determinations).

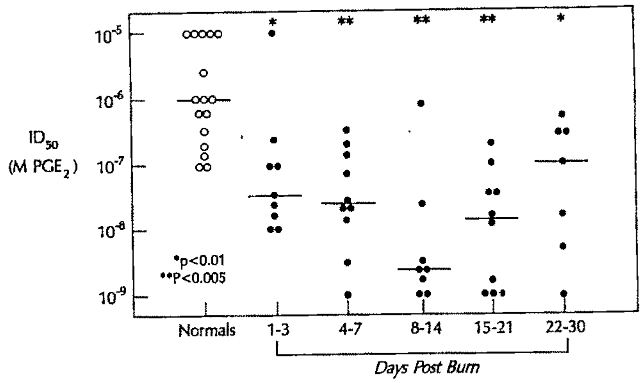

FIG. 3. Increased sensitivity by PBMC to PGE_2 after thermal injury. The ID_{50} concentration of PGE_2 was determined for normal controls (○) and burn patients (●) up to 30 days after injury. Horizontal lines in each group represent the median value of that group. Burn patients' PBMCs were found to be significantly more sensitive to PGE_2 than normal controls at every time point up to 30 days. Maximal sensitivity appeared to be at 8 to 14 days after injury.

Increased Sensitivity of Burn Patients' PBMCs to Inhibition by PGE_2

To investigate further the role of PGE_2 in the immunosuppression of burn patients, we determined the sensitivity of their PBMCs to inhibition by PGE_2 in the presence of Indo, as described above. In this study we examined 12 burn patients (mean age, 35 years; range, 22 to 65 years; TBSA, 45%; range, 20% to 90%) and compared them to 17 normal controls (mean age, 32 years; range, 22 to 61 years). The data are illustrated in Figure 3. The results indicate that at all time periods, the patients' PBMCs were significantly more sensitive to inhibition by PGE_2 than those of normal controls, with the maximum sensitivity occurring 8 to 14 days post burn. We also examined the relationship between burn size and PGE_2 sensitivity and found that PMBCs from patients with 40% or more TBSA burns were significantly ($p < 0.05$) more sensitive to the inhibitory effects of PGE_2 than those from patients with less than 40% TBSA burns (Fig. 4). Because the patients who developed clinically evident sepsis were also the patients with the biggest burns, it was difficult to determine which of these factors, burn size or sepsis, was

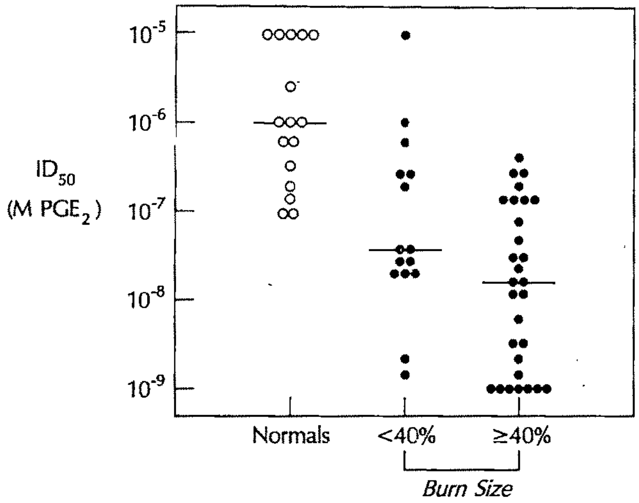

FIG. 4. Sensitivity to PGE_2 of PBMCs from patients with large *versus* small burns. Patients were divided into two groups: those with burns of 40% or more TSBA and those with less than 40% TBSA burns. The horizontal line indicates the median for each group. PBMCs from patients with 40% or greater TBSA burns were significantly ($p < 0.05$) more sensitive to PGE_2 than those patients with less than 40% TBSA burns. Both patient groups were significantly more sensitive to PGE_2 than normal controls.

more important in determining the PGE_2 sensitivity of the PBMCs from burn patients.

We also examined the relationship between suppressed mitogen responses and increased sensitivity to PGE_2. Prostaglandin E_2 sensitivity data were available on 13 patients' PBMCs whose PHA responses were suppressed more than 30% as compared to normal controls. Nine of these thirteen patients had ID_{50} values of 10^{-8} mol/L PGE_2 and the remaining four all had ID_{50} values of 10^{-7} mol/L PGE_2. These findings demonstrate that the increased sensitivity to PGE_2 may be an important cause of the suppression of mitogen responses seen in burn patients. However no direct correlation between suppression of the mitogen response and sensitivity to PGE_2 was found in PBMCs from burn patients ($r = 0.17$) or normal controls ($r = 0.01$).

Abrogation of PGE_2-mediated Suppression by IL-2

To determine whether PGE_2 functioned by inhibiting IL-2 production, we attempted to reverse the suppression of the mitogen response caused by PGE_2 by the addition of IL-2. A supraoptimal dose of purified human IL-2 (8 units/mL) was added to PBMC cultures that contained 10^{-7} mol/L PGE_2, 1 µg/mL Indo (to block *de novo* synthesis of PGE_2), and 6 µg/mL PHA, and these cells were assayed for mitogenic activity. The responses of the cultures containing PGE_2 with or without IL-2 were compared to those of cultures containing Indo and PHA only. The data are presented in Figure 5. Peripheral blood mononuclear cells from patients were significantly more suppressed by PGE_2 ($p < 0.005$) when compared to normal controls, again illustrating the increased sensitivity of burn patients' PBMCs to PGE_2. With the addition of IL-2, the PGE_2-mediated inhibition of the mitogen response in both normal controls and burn patients was totally reversed. This suggests that PGE_2-mediated suppression of lymphocyte activation in burn patients results primarily from the inhibition of IL-2 production by PGE_2.

PHA Responsiveness and IL-2 Production by PBMC from Endo-treated Volunteers

Volunteers were studied before (0 hours) receiving an intravenous infusion of *E. coli* endotoxin (4 ng/kg) and at 4 and 24 hours after Endo infusion. Peripheral blood mononuclear cells were harvested and studied for their responsiveness to PHA and the ability to produce IL-2 in response to PHA stimulation. Endo-treated volunteers were compared with those receiving infusions of saline alone. As noted in Figure 6, 4 hours after Endo infusion there was a significant inhibition of the ability of treated individuals' PBMCs to respond to PHA. This was accompanied by a simultaneous inhibition of production of IL-2 by PBMCs in response to PHA. Both these effects had returned to normal by 24 hours after Endo infusion.

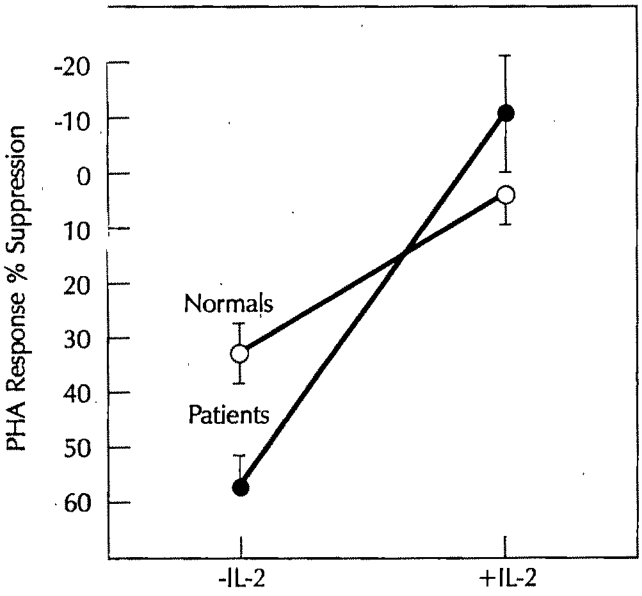

FIG. 5. The effect of the addition of IL-2 to cultures of burn patient PBMCs. Purified IL-2 was added to a PHA-induced blastogenesis assay that contained 10^{-7} mol/L PGE_2 and Indo. The vertical axis represents the percentage of the mitogen response in comparison to cultures that contained Indo only (0% suppression). The open circles represent the cultures of normal PBMCs and the closed circles represent PBMCs from patients. The values are the mean ± SEM.

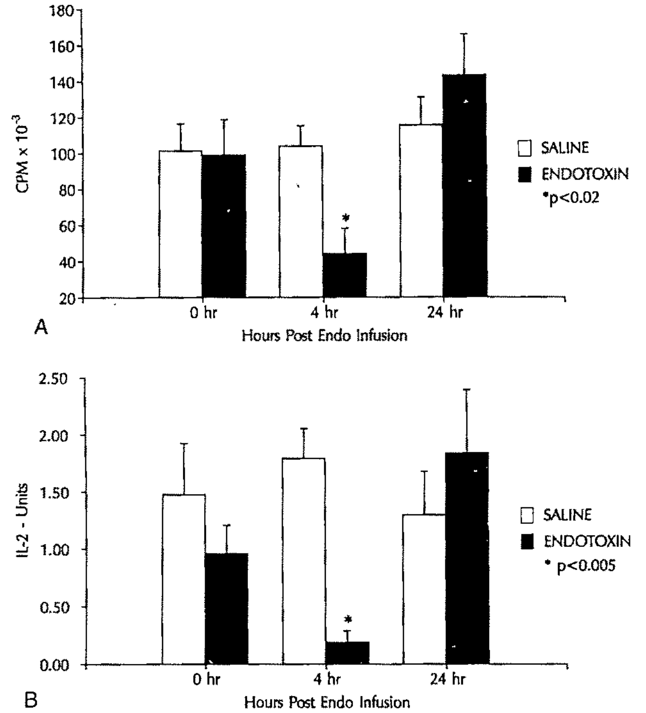

FIG. 6A and 6B. Effect of endotoxin infusion on PHA response and IL-2 production. PBMCs were harvested from volunteers treated with saline (■) or Endo (☐) and studied for (A) PHA response and (B) IL-2 production. Four hours after Endo infusion there was a significant ($p < 0.02$) inhibition of the PHA response ($p < 0.002$) and IL-2 production ($p < 0.005$), which returned to normal by 24 hours.

Sensitivity of PBMC from Endo-treated Volunteers to PGE$_2$

To determine the effect of Endo infusion on the sensitivity of PBMCs to exogenous PGE$_2$, PBMCs from Endo-treated volunteers were studied for their sensitivity to inhibition by graded dosages of PGE$_2$ in their responsiveness to PHA in experiments similar to those reported above for burn patients. Again (Fig. 7) in these experiments PBMCs from Endo-treated volunteers were markedly more sensitive to inhibition *in vitro* by PGE$_2$ 4 hours after Endo infusion. Saline infusion alone produced no such effect.

The Effect of Indo and IL-2 on the PHA Response of PBMCs from Endo-treated Volunteers

To determine whether Indo or exogenous IL-2 could restore the proliferative response of PBMCs to PHA, PBMCs obtained 4 hours after Endo infusion were cultured with PHA with or without the addition of Indo (1 μg/mL) or a supraoptimal dose (10 μ/mL) of recombinant human IL-2 (rhIL-2). The results shown in Figure 8 indicate that Indo partially and IL-2 totally restored the proliferative response to PHA of PBMCs harvested 4 hours after Endo infusion. The addition of exogenous rhIL-1 had no effect.

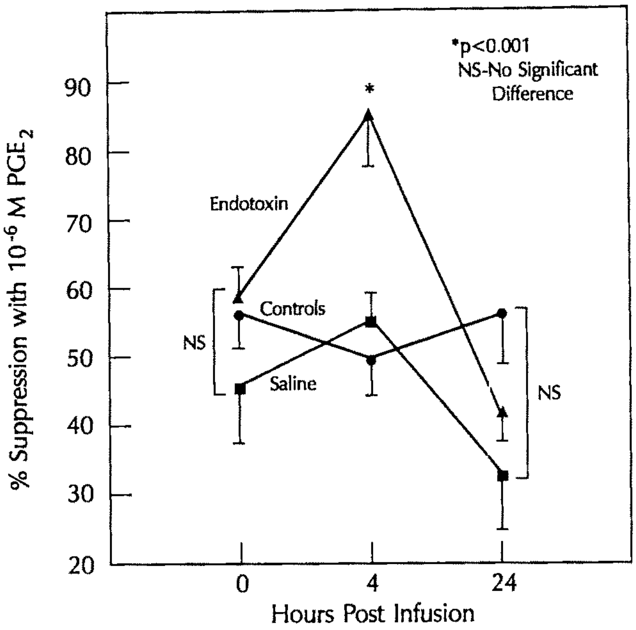

FIG. 7. Effect of Endotoxin infusion on the sensitivity of PBMC to PGE$_2$. PBMCs were harvested from volunteers treated with saline (■), endotoxin (▲), and normal controls (●) and studied for their inhibition by PGE$_2$ during response to PHA. PBMC from Endo-treated volunteers were more sensitive to inhibition by PGE$_2$ 4 hours after Endo infusion ($p < 0.001$).

Effect of Endo Infusion on PGE$_2$ Production by Adherent Cells from the PBMC Population

Peripheral blood mononuclear cells obtained from Endo- or saline-treated volunteers were allowed to adhere to plastic for 1 hour. Nonadherent cells removed by washing the adherent cells were stimulated in serum-free medium with *E. coli* endotoxin and the production of PGE$_2$ was measured by determining PGE$_2$ concentration in the culture supernatant by radioimmunoassay. The results shown in Table 1 indicate that PGE$_2$ production was maintained early after Endo infusion and had increased

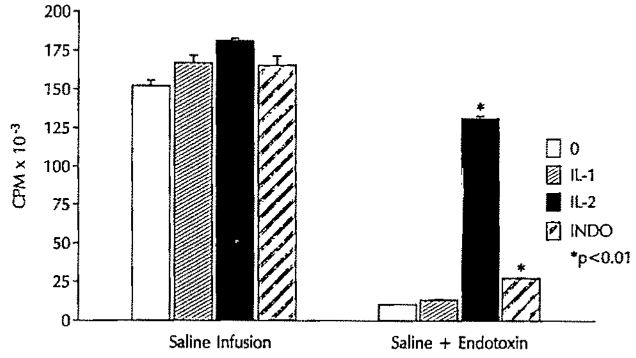

FIG. 8. Effect of indomethacin, IL-2, and IL-1 on the PHA response after endotoxin. PBMCs obtained 4 hours after saline or endotoxin infusion were cultured with PHA with or without the addition of indomethacin, IL-2, or IL-1. Results are shown for one representative experiment of six. Indomethacin partly and IL-2 completely restored the PHA response. IL-1 had no effect. * = $p < 0.01$, compared with 0 addition.

TABLE 1. *PGE_2 Synthesis*

	Infusion 0 (hours)	Time After Infusion (hours) 4	24
Saline	5656 + 1845	2679 + 621	3471 + 525
Endotoxin	5043 + 641	4364 + 1423	9257 + 2857*
Ibuprofen +Saline	5123 + 2376	4654 + 1451	3717 + 781
Ibuprofen +Endotoxin	6094 + 2567	3750 + 812	6975 + 782*

PGE_2 was determined in supernatants of LPS-stimulated adherent PBMC from normal volunteers at indicated times following infusion of saline, endotoxin, saline plus ibuprofen or ibuprofen + endotoxin.

* $p < 0.05$ compared with control infusion at same time.

to supranormal levels 24 hours later. Saline infusion produced no such effect.

Effect of Ibuprofen In Vivo *on PBMC Responses of Endo-treated Volunteers*

In a final series of experiments, six volunteers received 800 mg ibuprofen orally 2 hours before and at the time of infusion with Endo or saline. Peripheral blood mononuclear cells from these volunteers were tested for PHA response, IL-2 production, and adherent cells for PGE_2 production as above. It can be seen from Figure 9 that ibuprofen pretreatment abrogated the depression of the PHA response of PBMCs following Endo treatment. It also partially (but significantly) corrected the inhibition of IL-2 production following Endo infusion. Finally ibuprofen pretreatment blunted but did not entirely prevent the excessive PGE_2 production by adherent PBMCs noted 24 hours after Endo infusion (Table 1).

Discussion

Our laboratory has previously shown decreased IL-2 production by PBMCs from burned[4] and traumatically injured[5] patients despite normal or increased IL-1 production by adherent mononuclear cells from these patients. Prostaglandin E_2 has been shown to be a potent inhibitor of IL-2 production[6,7] and enhanced production of PGE_2 by monocytes obtained from burned patients has been demonstrated.[10] In addition increased levels of PGE_2 have been reported in both the burn wound and in the adjacent lymphatics,[8,9] as well as in the serum of burn patients.[13] In this study we examined the role of PGE_2 in the decreased T-cell responses and IL-2 production we have observed in burn patients.

Our results using the cyclooxygenase inhibitor indomethacin to enhance depressed mitogen responses and IL-2 production strongly suggest that PGE_2 does play a significant role in modulating the immune responses of PBMCs obtained from burned patients. These findings correlate with those of Faist et al.,[12] who showed that indomethacin was able to enhance the PHA response of PBMCs from patients with traumatic injury who demonstrated a depressed immune function. However indomethacin did not have a significant effect on PBMCs from normal controls or trauma patients who did not exhibit depressed mitogen responses. On the other hand, Antonacci et al.,[10] using the cyclooxygenase inhibitor WY-18251, found no increases in the autologous mixed lymphocyte reaction of burn patients 5 to 7 days after burn, although this work did show that monocytes from the same patients produced greatly increased quantities of PGE_2.

In the present study we have shown that the administration of a single dose of endotoxin to normal human volunteers can reproduce many of the abnormalities of lymphocyte function noted in the burn patients. These include diminished responsiveness of circulating lymphocytes to the T-cell mitogen PHA and diminished production of IL-2, in response to PHA stimulation. We also noted increased production of PGE_2 by adherent cells in the PBMC population. These findings lend further credence to the idea that many of the abnormalities in immune responsiveness that accompany serious injury are triggered by bacterial endotoxin leaked from the gut or released from enteric organisms translocated from the gut.

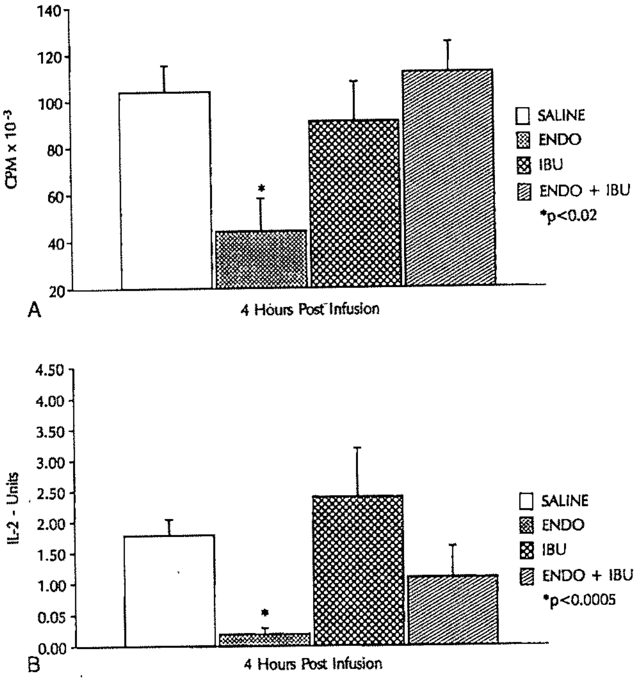

FIG. 9A and 9B. Effect of ibuprofen *in vivo* on PBMC responses after endotoxin. PEMCs obtained from volunteers who received either saline, endotoxin (Endo), or ibuprofen (IBU) before and at the time of saline or endotoxin infusion were studied for (A) PHA response and (B) IL-2 production. Ibuprofen pretreatment abrogated the depression of the PHA response and partially corrected the inhibition of IL-2 production seen after endotoxin.

The fact that many of the abnormalities of PBMC function noted in endotoxin-treated volunteers could be abrogated by ibuprofen further highlights the role of prostanoid production in mediating these abnormalities.

We also demonstrated that PBMCs from burn patients were considerably more sensitive to the inhibitory effects of PGE_2 than PBMCs from normal individuals. Furthermore burn patients' PBMCs, which exhibited suppressed mitogen responses, tended to be most sensitive to the inhibitory effects of PGE_2. The finding of increased sensitivity to PGE_2 supports the work of Goodwin and colleagues,[14] who reported an increased sensitivity to PGE_2 by PBMCs of patients who had undergone cardiac surgery or maternal labor. However their patients' PGE_2 sensitivity reached a peak 1 day after the stress and returned completely to normal within 10 days. They concluded that physical stress could cause an increase in the sensitivity of lymphocytes to PGE_2 and this could account for the immunosuppression seen in these patients.

Our results indicate that lymphocytes from thermally injured patients had a more profound sensitivity to PGE_2 that lasted for a much longer time than those reported by Goodwin et al.[14] and reached a peak between 8 and 14 days after injury. This time period corresponds to the peak incidence of sepsis in our patients and sepsis may have played a role in the increased sensitivity to PGE_2 we observed. Cahill et al.[15] have shown that mice infected with *Salmonella enteriditis* had depressed IL-2 production that was associated with an increased sensitivity of lymphocytes to the inhibitory effects of PGE_2. Furthermore preliminary studies in our laboratory on three uninjured septic patients demonstrated that all of these patients' PBMCs had increased sensitivity to PGE_2.

There are several possible mechanisms for the increased sensitivity of PBMCs to PGE_2. First it may be due to the increased ability of any suppressive substance to inhibit further an already suppressed immune response. Our results show that there was no direct correlation between the amount of suppression seen in both the mitogen response and IL-2 production of normal controls' and burn patients' PBMCs and sensitivity of these PBMCs to PGE_2. Goodwin et al.[14] have also reported similar findings in their study of PGE_2 sensitivity and physical stress, which suggested that increased suppression of mitogen responsiveness does not cause the increased sensitivity to PGE_2. Many of our patients' PBMCs demonstrated an increased sensitivity to PGE_2 although their mitogen responses and IL-2 production were not suppressed.

A second potential cause for increased sensitivity to PGE_2 is an increase in the number of cells capable of responding to PGE_2. Fischer et al.[16] have shown that only 10% to 30% of the peripheral T cells in normal individuals have receptors for PGE_2 and these cells were shown to be responsible for PGE_2-mediated suppression. Presumably an increase in the proportion of the T cells bearing PGE_2 receptors would increase the sensitivity of the total PBMC population to PGE_2. Goodwin et al.[14] have shown an increase in the relative proportion of T cells bearing the receptor for the Fc gamma chain of immunoglobulin in patients following surgery and maternal labor who had demonstrated increased sensitivity to PGE_2. This finding suggested that changes in T-cell subsets could account for some of the increased sensitivity to PGE_2 in their patients. Changes in T-cell subsets, including an increase in T cells bearing the Fc gamma receptor, have been reported in burn patients[17] and, therefore, the possibility exists that these changes may account for the increases in PGE_2 sensitivity seen in our burn patients.

A third possible cause is the presence of serum factors or hormones, which could increase sensitivity to PGE_2. Thermal and traumatic injury as well as sepsis are all known to increase the production of a wide variety of acute-phase proteins and hormones. One class of hormones that has been shown to increase sensitivity of lymphocytes to PGE_2 is the glucocorticoids. Mendelson and colleagues[18] have shown that glucocorticoids were able to enhance the ability of PGE_2 to increase the intracellular production of cAMP in lymphocytes. In 1976 Berenbaum et al.[19] examined the hypothesis that the combination of corticosteroids and PGE_2 could account for the depressed cell-mediated immune responses seen in trauma and burn patients because both were known to be increased after injury. In their system cells from normal individuals were incubated with a wide range of steroid and PGE_2 concentrations. They showed that PGE_2 and corticosteroids were synergistic in their ability to suppress immune responsiveness as assayed by mitogen-induced blastogenesis. Galanaud et al.[20] have shown that lymphocytes removed from volunteers 4 hours after an intravenous injection of hydrocortisone were significantly more sensitive to the inhibitory effects of PGE_2 on the formation of anti-TNP plaques *in vitro*. This suggests that increases in *in vivo* concentrations of hydrocortisone could increase the sensitivity of lymphocytes to *in vitro* PGE_2.

This idea is supported by the present studies of normal volunteers receiving bolus endotoxin infusions. These individuals demonstrated increased PGE_2 production, decreased IL-2 production, and suppressed responsiveness to the T-cell mitogen PHA. These volunteers demonstrated a significant and temporary increase in the sensitivity by circulating PBMCs to inhibition by PGE_2 *in vitro*. As reported elsewhere,[21] endotoxin infusion in these volunteers led to a dramatic increase in serum cortisol levels. This might very well mimic the situation described by Gallanaud et al.[20] and Berembaum et al.[19] and could account for the increased sensitivity of circulating T cells to PGE_2. Pretreatment of the volunteers with ibuprofen was shown to block the *in vivo* increase in serum cortisol concentrations in addition to inhibiting PGE_2 production, and thus ibuprofen may have abrogated any increased sensitivity of T cells to PGE_2 caused by cortisol.

Our *in vitro* data would tend to support the use of nonsteroidal anti-inflammatory drugs in burn victims and would argue for the addition of such agents to therapeutic trials of biologic agents directed at improving immune responses in seriously injured patients. Others have reached similar conclusions. Hansbrough and colleagues[22] have shown in a burned mouse model that the cyclooxygenase inhibitor, ibuprofen, was capable of restoring a contact sensitivity response to previously anergic animals as well as restoring decreased T helper/T suppressor ratios to normal in the spleens of these animals.[23] Furthermore they demonstrated that ibuprofen was capable of preventing the death of these burned mice following a septic challenge by cecal ligation and puncture.[24] Faist et al.[25] have demonstrated improved cellular immunity in patients receiving indomethacin after major surgery. Results from our laboratory[11] have demonstrated the involvement of PGE_2 in suppressing the production of IL-2 in thermally injured mice and that decreases in IL-2 production are associated with increased deaths following a septic challenge.[26] Furthermore we recently demonstrated that a combination of low-dose IL-2 plus low-dose indomethacin *in vivo* can increase significantly survival in this burn model.[27]

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DISCUSSION

DR. JONATHAN L. MEAKINS (Montreal, Quebec, Canada): From this excellent study, there are several questions that present themselves, and in the manuscript of over 20 pages I cannot cover all of the issues that come to mind, and so I will address basically three things.

In light of the fact that we have often thought that all patients had defects or at least some degree of the expression of these defects, it seems to me that one of the forward steps taken here is the identification that really only some patients have the full expression of the defect in host defenses. So that although we see that all peripheral blood monocytes in burn patients have some increased sensitivity to PGE_2, it is really only a smaller number of patients studied at specific times that seem to express this, and it is measured in a very strict manner; that is, the definition of abnormality is more than 2 standard deviations below the mean for controls.

Now I believe this must be a sequential study in the sense that I could not tell what the exact dates for inclusion of all the patients were, so that I would ask if the set of eight patients who had the 14 abnormalities in phenylalanine (PHA) responsiveness are a subset of the 10 patients with the changes in interleukin-2 (IL-2) synthesis. That is, do both defects occur concurrently or are they sometimes occurring in separate patients at different times?

The endotoxin data, a second point that I would like to discuss, demonstrates a second route to the production of a very similar, if not identical, form of immunosuppression.

In the manuscript, for which I thank Dr. Mannick, infection is able—that is, infection separate from trauma—to produce these same changes; that is, an increased sensitivity to PGE_2, decreased PHA responsiveness, and a decreased production of IL-2. Therefore we have infection; we have burn injury itself, sometimes; burn plus infection, and then fourthly, endotoxin; all producing similar changes.

Do they create these defects all by the same mechanism, not specifically the PGE_2 component; but is the cell production of PGE_2 the same, or are there different mechanisms of activation of this immunosuppression?

The last point comes back to the beginning, and that is that not all defects appear to be present in all patients all the time. Therapy therefore must be integrated with the presence of defects. Do you think we can identify these defects at the bedside? That is, can we tailor therapy at the time when the patients really need support of their immune system and can we tailor therapy on the principle that we should not mess with Mother Nature, or, as Osler would have told us, *primum non nocere?* So that in the use of indomethacin or the adjuvant use of IL-2 or other molecules or products that are or will be available to us soon, how do we identify the key time at which these molecules should be presented to the patient and when we should give them specifically, rather than use indomethacin as a global form of therapy for post-traumatic immunosuppression?

Dr. C. James Carrico (Dallas, Texas): What we have seen is that PGE_2 decreases the response of lymphocytes to a known mitogen, and we have seen it decreased by IL-2 production. We have heard the conclusion that this is suppression of the immune response system, and that suppression needs to be treated by cyclooxygenase or by some other mechanism of decreasing the PGE_2 production.

How do we know whether this is pathologic suppression of the immune system or physiologic down-regulation of the immune system? We think of the immune system appropriately as our defense against invaders and bacteria. Lewis Thomas warned us some time ago that it is so powerful that sometimes we may be in more danger from our immune system than from the invaders. And there is growing evidence that the multiple organ failure syndrome is really the immune system out of control.

Dr. Mannick, can you assure us that maybe you are not beginning to outline the mechanisms of down-regulation and that we ought to be pursuing this as a potentially beneficial effect, rather than corrected as a detriment?

Dr. Lewis M. Flint, Jr. (New Orleans, Lousiana): I have three questions for Dr. Mannick. Some of them stem from questions that have been asked by other discussants and others come from the fact that, at least as of my telephone call home yesterday afternoon, there were six patients with florid multiple systems organ failure in our Charity Hospital intensive care unit, and I would like some clinical guidance.

The first question is: Were there any clinical issues that could be identified that help to separate the patients whose cells were suppressed as opposed to those patients whose cells were not suppressed?

The second question has to do with potential methods for generic cyclooxygenase inhibition in these patients. In a limited clinical trial in patients with adult respiratory distress syndrome (ARDS) in our unit, we found that it was necessary to use a continuous infusion of the cyclooxygenase inhibitor over a relatively long period to get any effect. And I wonder if this is necessary, particularly in light of the fact that cyclooxygenase inhibition can cause some adverse effects, most notably renal vasoconstriction and redistribution of renal blood flow.

And finally if cyclooxygenase inhibition is going to be used as a therapeutic tool, how will we know when to use it and how will we know when to stop?

Dr. Basil Pruitt (Fort Sam Houston, Texas): Dr. Mannick, I enjoyed your paper very much and found a most interesting observation that the maximum immunosuppression was between the 8th and the 14th post-injury day, the most common time to diagnose infection. That emphasizes Dr. Meakins' question whether these changes were the cause or the effect of infection because of the difficulty in relating the diagnosis or onset to the manifestations temporally.

We also know that lymphocyte and white cell function can be changed by antibiotics and by hormones, so were these changes correlated with any antibiotic therapy in particular, or any significant changes in hormone level, such as the catacalls, in particular?

Dr. John Daly (Philadelphia, Pennsylvania): I have two questions. There are pathways that other investigators have looked at, one of them being that of nitrous oxide production by suppressor macrophages. I wonder if you have looked at all at any inhibitors *in vitro* of nitrous oxide production in your patients and whether there is any effect on lymphocyte function?

The second question is, can you determine why some patients have this excessive PGE_2 production, and others, presumably with the same degree of injury, do not?

Dr. Haile T. Debas (San Francisco, California): Just recently I heard a very elegant piece of work presented in which a naturally occurring receptor, specific receptor antagonist to IL-1, for example, has been cloned, and it specifically suppresses all the effects of endotoxin. It seems to me—and this comes from Dr. Mannick's neck of the woods—that the use of these specific receptor blockers may be a more direct way to deal with the problem than the use of cyclooxygenase inhibitors.

Dr. John A. Mannick (Closing discussion): The point brought out by several discussants is why did some of these patients get suppressed and others did not? I honestly do not know. I think that to some degree we can show that suppression is more likely to take place if the burn is big and if the patient gets infected. And that, I think, covers several of the questions that were asked.

The lesser burn patients, however, sometimes are suppressed, and patients are also sometimes suppressed even though they have no clinical evidence of being infected at that time. Obviously we have to rely to some degree in our thinking here on animal models, and it is very clear that in animal models of injury, improved survival can be obtained if one combines a cyclooxygenase inhibitor, apparently damping down the excessive production of prostaglandins by macrophages or monocytes, coupled with something that will stimulate T lymphocytes.

I think that the key to therapy here, and it probably can be done in a number of ways, is going to be modulating rather than eliminating the excessive mediator production by monocytes/macrophages. I quite agree with Dr. Carrico that Mother Nature is still pretty smart, but I think we also know that after serious injury, Mother Nature can kill you with cytokine poisoning, and we have to try to do something to reduce things back to a normal state of equilibrium.

There are probably a number of approaches to this, and Dr. Debas brings up the idea of the newly characterized interlukin-1 receptor antagonist that shows great promise in reducing some of the effects of endotoxin, for example, and also has been shown to be beneficial in septic shock in some animal preparations. I think that clinical trials are going to be the important way of solving these issues. We have animal models that point the way toward appropriate clinical trials, but we need prospective, randomized trials in our intensive care units, and in our burn units, to define what is safe and what is not, what is beneficial and what is not. We should now try to select first what appears to be both safe and beneficial for use in these clinical trials.

I am not convinced that cyclooxygenase inhibition if it is not pushed to ridiculous extremes is all that unsafe, so I still come down on the side of recommending clinical trials of the use of this kind of modulation of hyperactivity of macrophages.

Immunomodulatory Therapy With Thymopentin and Indomethacin

Successful Restoration of Interleukin-2 Synthesis in Patients Undergoing Major Surgery

EUGEN FAIST, M.D.,* ANDREAS MARKEWITZ, M.D.,† DIETMAR FUCHS, M.D.,‡ STEFAN LANG, M.S.,*
SUSANNE ZARIUS, B.S.,* FRIEDRICH-WILHELM SCHILDBERG, M.D.,* HELMUT WACHTER, M.D.,‡
and BRUNO REICHART, M.D.†

Prostaglandin E_2 (PGE_2)-mediated monocyte (Mφ) suppressor activity and inadequate T-helper cell function represent the mechanistic keystones of trauma-induced impairment of cell-mediated immunity (CMI). In a prospective randomized trial, the immunorestorative potential of a combined therapy with the thymomimetic substance Thymopentin (TP-5; Timunox®, Cilag GMBH, Sulzbach, FRG) and the cyclooxygenase inhibitor indomethacin (Indo) in 60 patients (mean age, 63 ± 2 years) undergoing open heart surgery was studied. Perioperative immunologic screening was carried out on days −2, 3, 1, 5, and 7 and included the *in vivo* delayed type hypersensitivity (DTH) skin response, phenotyping for peripheral blood mononuclear cell (PBMC)-specific and nonspecific induction of lymphoproliferative responses, *in vitro* interleukin-2 (IL-2) synthesis, as well as the serum concentration of D-erythro-Neopterin (NPT) and of gamma interferon (γ-IFN). The study protocol comprised three groups (n = 20): PA (Indo 150 mg administered intravenously on days 0 to 5), PB (TP-5 administered subcutaneously on days 0, 2, 4, and Indo), and PC (control). In contrast to PC, significant immunorestoration could be demonstrated in PB, as DTH scores on day 7, as well as proliferative responses in cell cultures were not depressed after operation ($p < 0.05$). Cell-surface receptor expression for the CD3+, CD4+, and IL-2 receptor-positive (IL-2R+) lymphocyte subpopulations following surgery was reduced to 75% of baseline values in PC, while in PB, receptor protection for CD4+ and IL-2R+ subpopulations (more than 15% above baseline) was observed. Interleukin-2 synthesis (average baseline value, 0.7 + 0.08 U/mL) in cell cultures of PC was massively suppressed, with lymphokine concentrations in the supernatants never more than 0.27 ± 0.05 U/mL. In PA cultures, IL-2 synthesis was impaired as well but not as precipitously as in PC. In contrast, in PB cultures, the average IL-2 production on consecutive postoperative days was never below baseline values. This study clearly demonstrates that the combined Indo/TP-5 therapy is superior to single Indo administration and can adequately preserve and/or restore intact MφT-cell interaction and thus appears to be a feasible approach to maintain normal host defense activity in traumatized individuals.

From the Departments of Surgery and Cardiac Surgery,† Ludwig-Maximilians-University, Klinikum Großhadern, Munich, Federal Republic of Germany; and the Institute of Medical Chemistry and Biochemistry,‡ University of Innsbruck, Innsbruck, Austria*

PATIENTS UNDERGOING CARDIAC surgery with extracorporeal circulation (ECC) are subjected to an immediate massive impact on their host defense integrity due to the combined effect of tissue trauma, multiple blood transfusions, and a whole-body inflammationlike state, induced through the extensive contact between blood and foreign material. The resulting impairment of cell-mediated immunity (CMI) has its clinical correlate because there is enhanced susceptibility of these patients to infectious complications.[1-3] In our own institution, during the year 1990, 55% of the deaths in patients undergoing ECC were due to septic multiple-organ failure.

For the reasons outlined above, it can be suggested that the major cardiac surgery patient can serve as an excellent *in vivo* model to investigate the efficacy of therapeutic strategies designed to counteract the impairment of immunomechanistics induced through severe trauma. We and others have demonstrated[4-6] that the alteration of CMI following trauma is mainly due to the disruption of intact monocyte (Mφ)/T-cell interaction. Within this phenomenon we see a shift of the cell ratio in the compartment of peripheral blood mononuclear cells (PBMC), with a considerable increase of prostaglandin E_2 (PGE_2) synthesizing Mφ and a simultaneous decrease of functionally competent CD3+ and CD4+ lymphocytes. T-cell dysfunction in states of profound stress is characterized by impaired synthesis of two crucial cytokines—in-

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Address reprint requests to Eugen Faist, M.D., Ludwig-Maximilians-University, Department of Surgery, Marchioninistraße 15, D-8000 München 70, Federal Republic of Germany.

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terleukin-2 (IL-2) and gamma interferon (γ-IFN).[7,8] The inability to produce adequate amounts of IL-2 results in incomplete proliferative T-cell responses to antigenic stimuli, while a lack of γ-IFN results inefficient Mϕ antigen presentation. It has been demonstrated that both defects are keystones of suppressed CMI function following trauma, with subsequent development of sepsis.[9-11]

The information derived from the dissection of downregulatory mechanisms responsible for the development of injury-related immunoincompetence provided the incentive for the development of therapeutic regimens designed to prevent a major collapse of CMI.

The use of two classes of substances seemed to be most suitable for immunoprotection and/or immunorestoration: first nonsteroidal anti-inflammatory drugs, which block immunoreactive PGE_2,[12] the common link of malfunction of the Mϕ/T-cell interactive network in states of trauma; and second the synthetic thymomimetic pentapeptide Thymopentin (TP-5; Timunox®, Cilag GMBH, Sulzbach, FRG), with its crucial characteristics—restoration of immunobalance, T-cell activation, and acceleration of T-cell recruitment.[13]

We have conducted a number of clinical trials in recent years to scrutinize the immunoaugmenting potential of TP-5 as well as of the cyclooxygenase inhibitor indomethacin (Indo) in patients undergoing major surgery.[14] A perioperative two-shot subcutaneous administration of 50 mg TP-5 in patients undergoing open heart surgery resulted in a restoration of the *in vitro* lymphocyte proliferative responses, as well as in the delayed type hypersensitivity (DTH) responses, compared to a placebo-treated control population. It was disillusioning to recognize, however, that T-cell receptor protection (CD3+, CD4+) and restoration of IL-2 synthesis could not be achieved with that treatment modality.[15]

Conversely postoperative administration of Indo in patients undergoing gastrectomy or reconstruction of the abdominal aorta resulted in an impressive protection of T-cell receptor expression for the CD3+, CD4+, and IL-2 receptor-positive (IL-2R+) subpopulations. This treatment also controlled overwhelming relative monocytosis. Furthermore the preservation of the preoperative *in vivo* DTH immunoreactivity, in contrast to untreated patients, could be demonstrated.[16] However restoration of depressed IL-2 synthesis could not be attained with Indo administration, which was in contrast to many *in vivo* experiments that showed that Indo restores depressed IL-2 production.[17]

Based on the findings in these single-agent studies and the knowledge that trauma induced depression of CMI represents a multimechanistic phenomenon, the protocol for a combined-agent therapeutic trial was designed. It was the objective of this prospective randomized study to quantify, specify, and compare the immunorestorative potential of a combined therapy with the cyclooxygenase inhibitor Indo and the thymomimetic substance Thymopentin *versus* single-drug administration of Indo following ECC.

Cell-mediated immunity parameters studied, *in vivo* and *in vitro*, included the DTH skin response to recall antigens, PBMC phenotyping, specific and nonspecific induction of lymphoproliferative responses, and *in vitro* IL-2 synthesis. In addition, for CMI serum markers, we evaluated the concentration of D-erythro-Neopterin, a sensitive indicator of Mϕ activation, as well as the concentration of γ-IFN as a marker for T-cell activation. The results indicate that simultaneous cyclooxygenase inhibition and T-cell activation greatly enhanced the forward regulatory axis of cell-mediated immune mechanisms following ECC.

Materials and Methods

Patient Population

Surgical patients in the surgical wards and the intensive care unit of the Department of Cardiac Surgery of the LM University Munich, Klinikum Groβhadern, were studied. This study was approved by the Medical Ethics Committee of the Faculty of Medicine.

From November 1, 1989 until October 31, 1990, studies were conducted in 60 patients (45 men, 15 women) whose average age was 63 ± 7 years. All patients had acquired or congenital heart disease and had to undergo ECC surgery with coronary artery bypass grafting or valve replacement (Table 1).

Experimental Protocol

For the prospectively randomized study (Fig. 1), patients were divided into three groups: group A patients (PA) (n = 20) were given indomethacin (Confortid®, Dumex, Denmark) in 100-mg intravenous doses immediately after surgery (D0), and 500-mg doses three time daily until day 5 after operation. Group B patients (PB) (n = 20) besides Indo therapy also received 50 mg of TP-5 (Tim-

TABLE 1. *Clinical Data (n = 60)*

Characteristic	
Average age (yr)	63 ± 7 (range, 55–78)
M/F	45/15
Surgical procedure	
CABG	n = 46
Valve replacement	n = 14

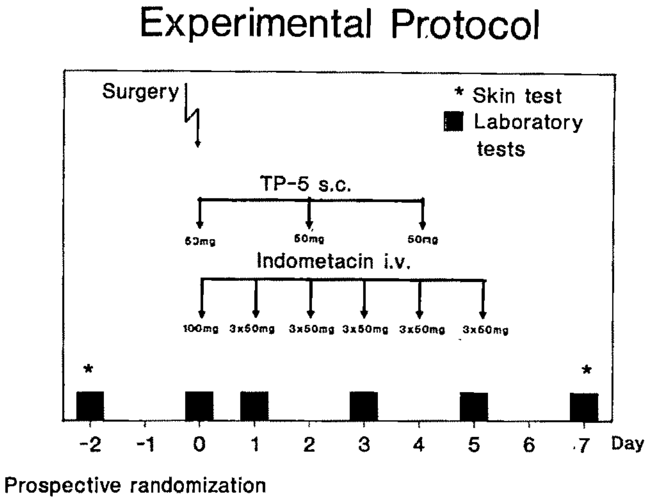


FIG. 1. Depiction of the experimental protocol, showing time of indomethacin and TP-5 administration and frequency of laboratory tests.

munox®, Cilag, FRG) administered subcutaneously 2 hours before operation and 48 hours and 96 hours after operation. Group C patients (PC) (n = 20) served as the control population, undergoing conventional intensive care unit therapy after operation.

Age, underlying disease, and quality of surgical procedure were highly comparable in all three groups.

Immunologic screening of the patients was carried out twice before operation, immediately after admission, and on the day of the operation, but was calculated as one preoperative value, as well as on days 1, 3, 5, and 7 after operation.

Cell Preparation and Culture

For lymphocyte studies 50 mL of peripheral blood was obtained in sterile heparinized tubes. The blood samples were diluted 1:2 in Hanks' buffered saline solution (Gibco Laboratories, Grand Island, NY) with 2% Penicillin-Streptomycin. Peripheral blood mononuclear cell isolation was performed immediately by standard Ficoll-Hypaque density gradient centrifugation at 1500 rpm at 4 C for 35 minutes. After resuspending the cells with 15% fetal calf serum (Gibco Laboratories), cell counts were performed with a hemocytometer using trypan blue exclusion as a test of viability. Viability always exceeded 95%. Appropriate cell suspension concentrations were then prepared for the different assays to be performed.

Mitogen Assay

At concentrations of 1×10^5 cells per well, PBMCs were added in triplicates to flat-bottomed, 96-well microtiter plates and antigen cocktail (AgC) was added to the wells at a final concentration of 50 mg/L. Antigen cocktail (Behring Co., Marburg, FRG) is a mixture containing five antigens—purified protein derivative, tetanus toxoid, streptolysin, mumps, and vaccinia antigen.

The cultures were incubated at 37 C in a 6% CO_2 incubator for 120 hours. Six hours before harvesting, the cultures were pulsed with 18.5×10^3 Bq per well of tritiated thymidine. The cultures were harvested on glass-fiber paper with a multiple-automated sample harvester. Vials, holding the filter strips and scintillation fluid, were counted in a beta counter. The net count per minute of triplicate cultures was calculated as the net count for the cells with AgC minus the net count for the cells without AgC. Lymphocyte proliferation was also performed with phytohemagglutinin (PHA), which was added to the triplicates at a final concentration of 0.5 μg/mL. These cultures were incubated for 72 hours before being pulsed with thymidine.

PBMC Phenotyping

Phenotyping of Ficoll-Hypaque preparations of mononuclear cells, as described elsewhere,[18] was performed using monoclonal antibodies to quantitate CD3+, CD4+, and CD8+ T cells (Becton-Dickinson, Heidelberg, FRG), LeuM3+ monocytes (Becton-Dickinson), as well as IL-2R+ cells (Biotest Diagnostics, FRG). The number of cells that were stained with the antibodies was assayed using fluorescence microscopy. The IL-2R antibody identifies the human IL-2R on mitogen- and antigen-activated T cells. Enumeration was performed after 48 hours incubation of the PBMCs with 1 mg/mL PHA.

IL-2 Generation and Activity Assay

Interleukin-2 was generated by culturing PBMC suspensions at a cell concentration of 20 PBMCs per liter in 5-mL culture tubes in the presence of highly purified PHA at a final concentration of 2.5 mg/L at 37 C, 6% CO_2. After 48 hours supernatants were collected and stored at −70 C until assayed.

The assay for the detection of IL-2 activity was a modification of the method described by Gillis et al.[19] Briefly frozen long-term cultures (5 days) of human T cells with concanavalin A blasts serving as IL-2–sensitive target cells were thawed, washed, and resuspended in Roswell Park Memorial Institute 1640 medium supplemented with 2% streptomycin-penicillin and 20% pooled human serum. Supernatants to be tested for the amount of IL-2 released on stimulation with PHA were placed in 100-μL serial dilutions from 1:2 to 1:128 in 96-well, round-bottomed microtiter plates in triplicate. Next 100 μL of concanavalin A blasts were added to each well, resulting in a final concentration of 4×10^4 cells per well. The IL-2 activity of the supernatants to be tested was compared with the activity induced by the IL-2 standard solution, which contained 1 U/mL by definition (Lymphocult HP, Biotest, Offenburg, FRG). Units of IL-2 were calculated by comparison with the cell proliferation induced with the Lymphocult test solution.

Assay for γ-IFN

Immunoradiometry was used (a gift of Centocor Inc., Malvern, PA) employing a modified application[20] that allows the detection of γ-IFN in sera with sufficient sensitivity. Beads with monoclonal antihuman γ-IFN antibody were incubated with 200 μL serum at room temperature for 16 hours. Then the beads were washed with 3 mL distilled water and incubated for an additional 16 hours with 200 μL of ^{125}I-labeled tracer. With this modified procedure, more than 80% of the analyte is bound to the solid-phase antibody.[20] Radioactivity was counted with a gamma counter (CliniGamma 1272; Wallac Oy, Turku, Finland). Gamma interferon activity is expressed as National Institutes of Health (NIH) units (U).[21] The limit of detection was 18 U/L.[20]

Assay for Neopterin

Neopterin was quantified by radioimmunoassay (RIAcid, Henning-Berlin, Berlin, FRG). Fifty microliters of serum were incubated with 100 μL of neopterin antiserum for 1 hour at room temperature, and 100 μL of ^{125}I-labeled tracer was added followed by incubation for 1 hour. Two milliliters of aqueous polyethylene glycol 6000 solution (60 g/L) was then added. After centrifugation at 2000*g* for 10 minutes, radioactivity was counted with the gamma counter. The detection limit was 1 nmol/L.[22]

DTH Skin Testing

The DTH response to seven recall antigens was carried out 2 days before surgery and on day 7 after operation. The recall antigens (tetanus, diptheria, Streptococcus, old tuberculin, Candida, Trichophyton, and Proteus mirabilis) and a glycerin control are contained in a test system (Multitest Mérieux, Hamburg, FRG). The antigens were applied on the volar side of the forearm by firm pressure with a mechanical applicator. The skin test was evaluated 48 hours after application. The final score consisted of the number of positive antigen reactions and the sum of the mean diameters of these reactions. A reaction smaller than 2 mm was considered negative.

Statistical Analysis

Statistical analysis was carried out using analysis of variance for intergroup comparison. Student's paired t test was used for intragroup comparison. Probability values less than 0.05 were considered significant.

Results

Phenotyping Studies

A significant persistent reduction of cell-surface receptor expression for the CD3+, CD4+, and IL-2R+ subpopulations following surgery compared to preoperative values was observed in PC (Fig. 2). The lowest level of depression was seen on day 1, with an average range of reduction for CD3+ and CD4+ leukocytes between 20% and 30%. Indo treatment resulted in a clearly demonstrable alleviation of depressed receptor expression in PA compared to PC. In PB, after day 1, an absolute receptor protection, partially with elevations toward supranormal ranges, especially for the CD4+ subpopulation 114% ± 4% (D3), 120% ± 4% (D5), 124% ± 3% (D7), was observed.

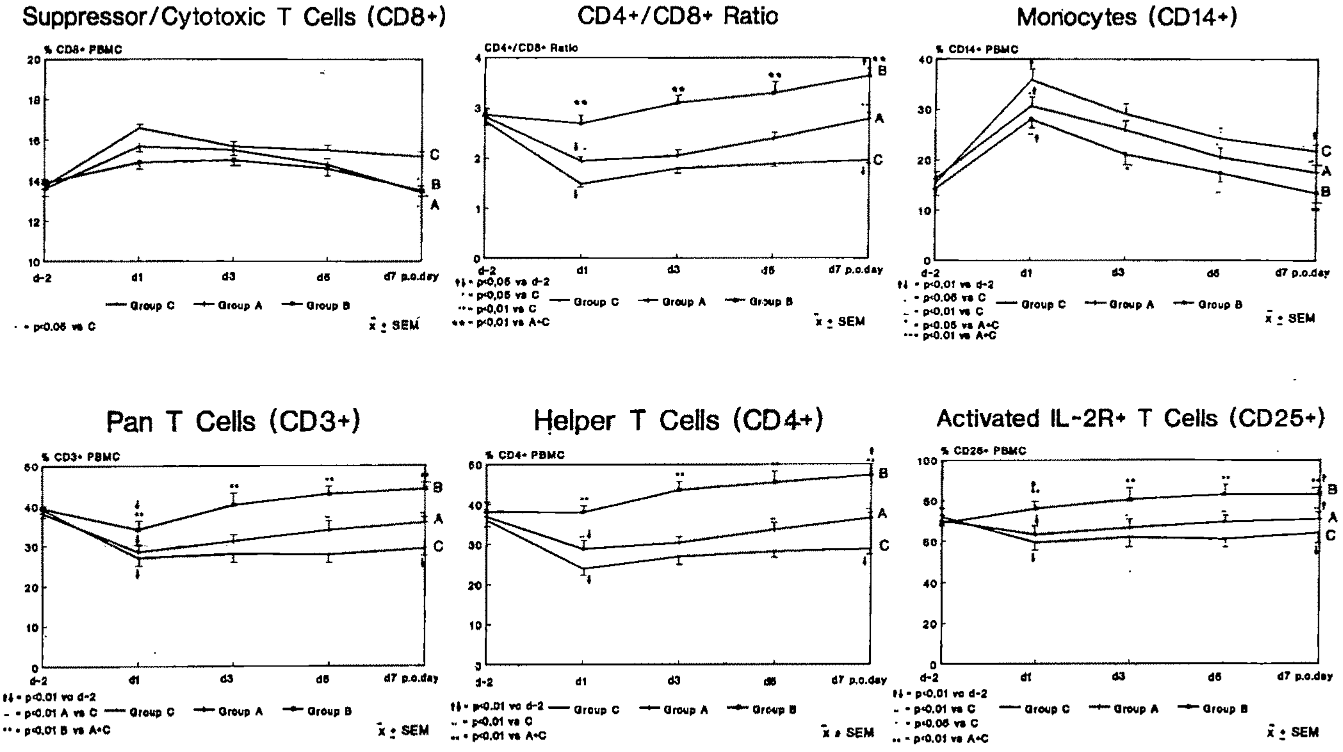
An adequate number of IL-2R+ (CD25+) cells could be maintained with combined therapy, as well as with sole Indo treatment, compared to a considerable receptor depletion in the PC group.

The baseline value of 14% ± 0.6% CD8+ cells was elevated on day 1 in all groups but returned to normal in PA and PB on day 7, in contrast to PC. When calculating the CD4+/CD8+ ratio on consecutive postoperative days compared to the average baseline value of 2.8 ± 0.2 for all groups, a clear gradation of values for the individual groups with crucial differences between PC *versus* PA and PB was observed. A striking difference appears on day 7 with a CD4+/CD8+ ratio (2.0 ± 0.1) in PC, which is still below baseline ($p < 0.05$), while there was a value (3.7 ± 0.2) for PB, expressing a significant overcorrection from baseline ($p < 0.05$)

Surgery resulted in a massive initial increase of CD14+ Mφ in all groups compared to the average baseline value of 15% ± 0.7% cells. On day 1 the number of Mφ within the PBMC population calculated ranged between 36% ± 2% (PC), 31% ± 2% (PA), and 28% ± 1% (PB). A parallel pattern of decreasing numbers was seen on consecutive days after surgery for all groups. However the initial significant differences in the Mφ counts in the postoperative intergroup comparison persisted until the end of the observation period. Thus on days 5 and 7 there was still a +61% ± 9% and +46% ± 9% elevation in PC, while in PA (+34% ± 9% [day 5], +13% ± 7% [day 7]), and PB (+29% ± 9% [day 5], −1.5% [day 7]) the counts were significantly lower.

Proliferative Responses

The preoperative average PHA-induced lymphocyte proliferation in all patient groups (31892 ± 1497 CPM [PC], 33159 ± 1504 CPM [PA], and 31548 ± 1412 CPM [PB]) was nearly identical. Furthermore the AgC-induced proliferative responses were similar: 17670 ± 605 CPM (PC), 18473 ± 719 CPM (PA), and 17503 ± 396 CPM (PB) (Fig. 3).



PBMC Phenotyping

Suppressor/Cytotoxic T Cells (CD8+)

CD4+/CD8+ Ratio

Monocytes (CD14+)

Pan T Cells (CD3+)

Helper T Cells (CD4+)

Activated IL-2R+ T Cells (CD25+)

FIG. 2. Phenotyping of peripheral blood mononuclear cell subpopulations on consecutive postoperative days, for the individual patient groups PA, PB, and PC. Changes in CD3+, CD4+, CD8+, and IL-2 R+ (CD25+) T cells, in the CD4+/CD8+ ratio and in CD14+ monocytes, marked with the monoclonal antibody LeuM3 are depicted. Asterisks indicate values that are significantly different ($p < 0.01$, $p < 0.05$) in intergroup and intertime (vsD-2) comparison.

PHA-induced proliferation in PB cultures was not depressed during the postoperative course, but showed mild elevations within the range of +17.8% (on day 3) and +23.6% (on day 5) compared to baseline.

In contrast proliferation values of PC and PA cultures were significantly suppressed ($p < 0.05$), reaching 58.2% ± 2.6% (PC) and 74.9% ± 2.3% (PA) of baseline levels.

For PC and PA, although a gradual recovery of proliferation was seen, the responses were still significantly lower on day 7 compared to preoperative values. There was also a significant difference between the proliferative responses of PA and PC on all postoperative days.

Compared to the average baseline values of specific antigen (AgC)-induced PBMC proliferation, a significant decrease on day 1 in all groups was observed. While PB proliferation was reduced to 84.3% ± 3.4% of baseline, PA (67.2% ± 2.9%) and PC (53.4% ± 3.9%) values decreased more substantially. There was a clear tendency toward normalization on consecutive days after surgery in PA and PC, without reaching the baseline level by day 7 ($p < 0.05$). In contrast proliferation of PB on day 5 was already up to 103%.

Interleukin-2 Synthesis

The preoperative values of PHA-induced IL-2 synthesis were comparable in all patient groups with 0.69 ± 0.05 (U/mL) (PC), 0.59 ± 0.04 (U/mL) (PA), and 0.60 ± 0.04 (U/mL) (PB) but were considerably lower than the control value (0.80 ± 0.04 U/mL) derived from healthy human volunteers (Fig. 4).

During the postoperative course, IL-2 synthesis in cell cultures of PC were massively suppressed, with lymphokine concentrations in the supernatants never more than 0.27 ± 0.05 U/mL (on day 3) with a nadir on day 7 (0.10 ± 0.02). In PA cultures a considerable impairment of IL-2 production compared to preoperative values was observed, although it was not as precipitous overall, as in PC. In PA cultures on day 7, the IL-2 production was as low as 29% (0.16 ± 0.02 U/mL) compared to preoperative values. In contrast in PB cultures the average IL-2 pro-

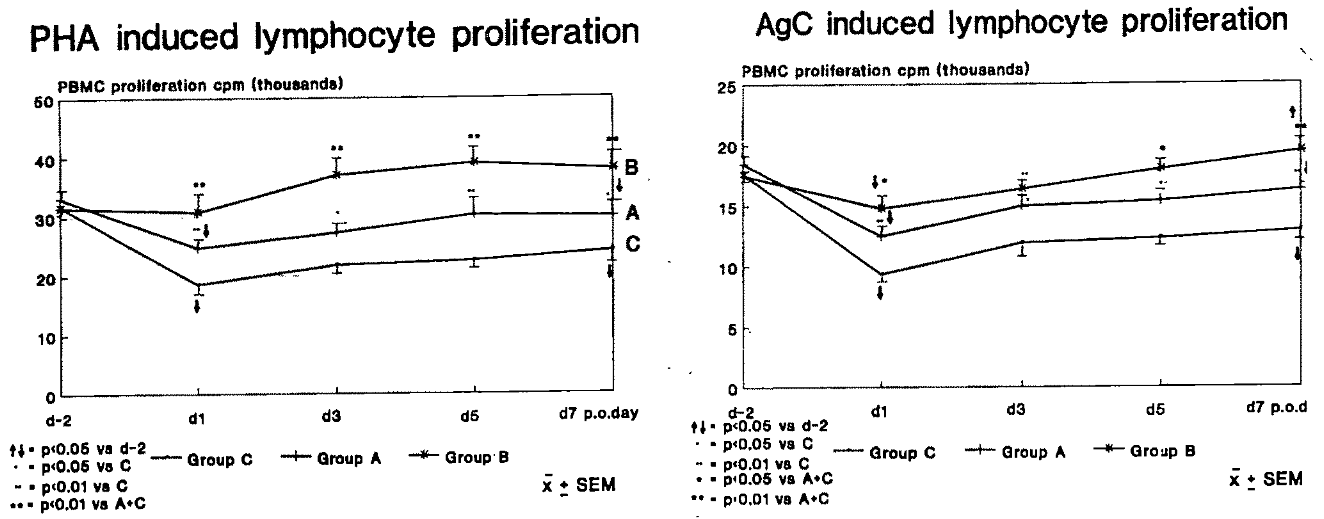


FIG. 3. Nonspecific-, PHA mitogen-induced, as well as specific antigen (AgC)-induced lymphocyte blastogenesis before operation (D-2) as well as on consecutive days after surgery for the three patient groups, is depicted. Asterisks indicate values that are significantly different ($p < 0.01$, $p < 0.05$) in the intergroup and intertime (vsD-2) comparison.

duction on consecutive postoperative days was never less than the baseline value, showing even slight elevation up to +18% (0.70 ± 0.08 U/mL) on day 5. On all days there was a significant difference between the IL-2 synthesis in PB *versus* PA and PC.

DTH Response

In PC the operative trauma resulted in a significant depression of quantity and quality of the skin responses on day 7 compared to the preoperative scores (Fig. 5).

Thus the average value of positive Ag reactions (3.1 ± 0.04) (100%) and the sum of mean diameters of the skin response (13.6 ± 1.4) (100%) decreased to 1.9 ± 0.3 (62% of baseline) and 7.4 ± 1.3 (54% of baseline), respectively. In PA the baseline values (3.0 ± 0.3+ reactions/ 11.4 ± 1.4 mean diameters) decreased to 2.4 ± 0.3 (80%) and 8.5 ± 1.4 (74%), respectively. In comparison to PC, PB values on day 7 were significantly higher ($p < 0.05$) (3.1 ± 0.4 [100%]/12.4 ± 1.7 [122%]) and did not fall

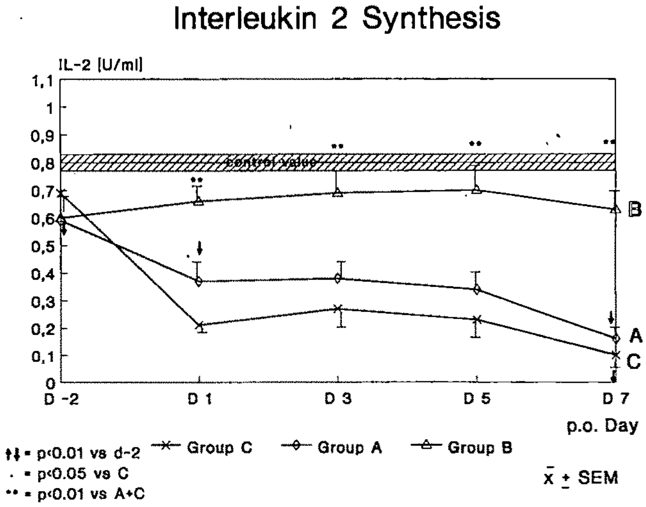


FIG. 4. PHA-induced *in vitro* IL-2 synthesis before operation (D-2) and on various days following operative trauma for the three patient groups studied. Horizontal bars express the IL-2 control value (0.8 ± 0.04 U/mL) in this laboratory derived from healthy laboratory volunteers. Asterisks indicate values that are significantly different ($p < 0.01$, $p < 0.05$) in the intergroup and intertime (vsD-2) comparison.

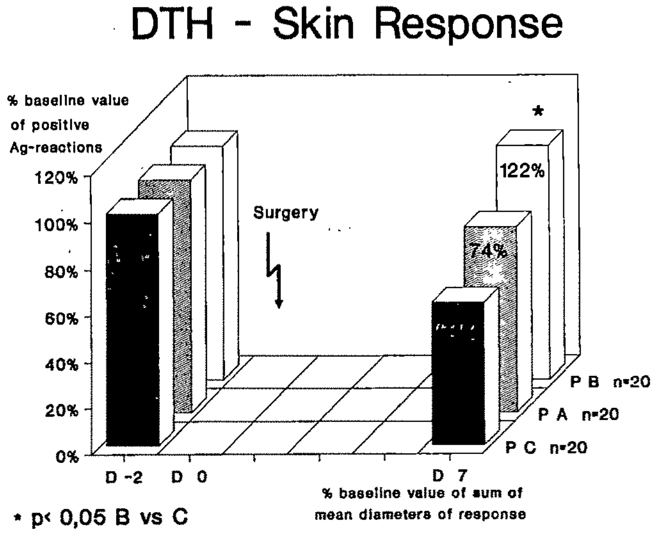


FIG. 5. DTH response skin testing was done before operation (D-2) and on D7 after operation. The score of the DTH response consists of the number of positive Ag reactions and the sum of mean diameters of the response. The different height of the individual bars indicates changes in the number of positive Ag reactions after operation. The percentage written on the bars indicate the changes of the mean diameters of response in the individual groups compared to baseline values.

short of their respective baseline values (3.1 ± 0.3/10.2 ± 1.2).

Serum Neopterin

The average preoperative NPT concentration in the patient population studied was 8.9 ± 0.9 nmol/L (nanomolar) (Fig. 6). In PC a very rapid increase of NPT in serum was seen on day 1 (20.8 ± 3.3 nmol/L), with a peak value of 29.1 ± 3.2 nmol/L on day 3. In PA (12.8 ± 6.8 nmol/L) and PB (14.2 ± 6.6 nmol/L) there was a much more subtle increase of this monocyte activation marker. On day 3 the NPT concentration in PA (27.6 ± 4.6 nmol/L) was almost identical to the peak value of PC, while in PB a concentration of about 21 nmol/L was measured, remaining at this level until day 5.

On day 7 NPT concentrations in all patient groups decreased to a level of about 19 nmol/L.

Serum γ-IFN

Serum γ-IFN concentrations in PB were continuously increasing from their preoperative day 2 value of 115 ± 15 U/mL on consecutive postoperative days to 129 ± 21 U/mL on day 1, 144 ± 28 U/mL on day 3, and 143 ± 17 U/mL on day 5, finally reaching 173 ± 49 U/mL on day 7 (Fig. 7). In contrast to these findings, the γ-IFN concentrations on day 1 in PA (105 ± 15 U/mL) and PC (103 ± 13 U/mL) remained within the range of their preoperative levels, then declined in parallel on day 3 to 82 ± 13 U/mL (PC) and 90 ± 11 U/mL (PA), respectively. While until day 7 in PA the preoperative mediator level was regained, γ-IFN concentrations in PC showed a further decrease to 64 ± 5 U/mL. The difference of γ-IFN serum concentrations between PC and PB, starting on day 3, were significant ($p < 0.05$).

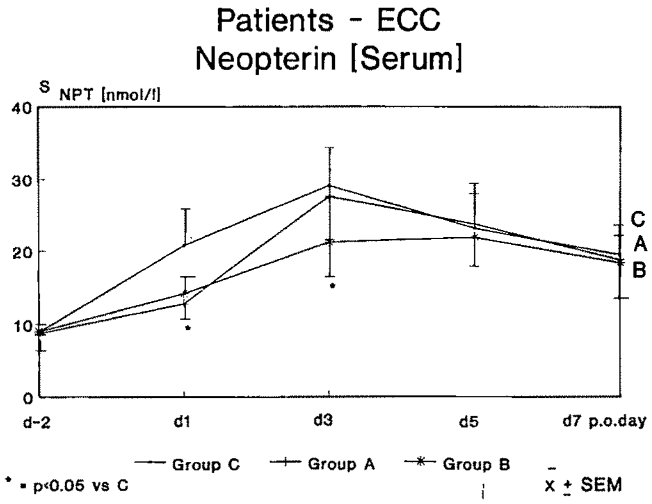


FIG. 6. Perioperative changes of serum NPT levels (nmol/L) in the individual patient groups. Asterisks denote values that are statistically different ($p < 0.05$) in the intergroup comparison.

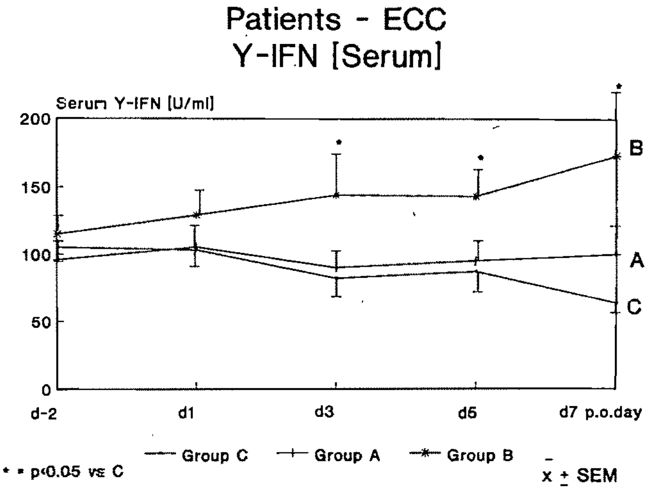


FIG. 7. Depiction of perioperative changes of serum γ-IFN levels (U/mL) in the individual patient groups. Asterisks denote values that are statistically different ($p < 0.05$) in the intergroup comparison.

Clinical Results

In this population of 60 patients, seven infectious complications (11.6%) were seen during the postoperative observation period of 20 days (Table 2). In group A there were two cases of major infectious episodes with clinical manifestation on day 7 (sepsis of unknown origin) and day 15 (pneumonia). The latter patient died with septic multiple-organ failure on day 20. In group B three infectious episodes became manifest. The origins of infection were the contamination of a central venous line, sternal osteitis, and cystitis, with no infection occurring before day 7.

In group C two infectious episodes were documented. In one patient sternal osteitis occurred on day 5. The second patient died from septic multiple-organ failure originating from pneumonia with clinical manifestation on day 5.

TABLE 2. *Clinical Results: Infections (n = 7)*

Group A n = 2	Group B n = 3	Group C n = 2
Sepsis (D7) Pneumonia (D15)†	Cystitis (D8) Sternal osteitis (D7) Central venous catheter (D7)	Sternal osteitis (D5) Pneumonia (D5)†

† Died.
D, day.

Discussion

This study shows clearly that combined administration of the cyclooxygenase inhibitor Indo and the thymomimetic drug TP-5 can optimally prevent the trauma-induced depression or breakdown of CMI mechanisms that normally occur in individuals after major operative and ECC injury. While corroborating some previous data derived from single-drug studies with these particular substances, this investigation, through a number of parameters, demonstrates the superiority of the immunoaugmenting effect of a combined Indo/TP-5 administration *versus* single-drug use.

The global assessment of *in vivo* (DTH) immunoreactivity in PB (Indo + TP-5) after operation indicated no difference from the preoperative status, in contrast to a significant reduction seen in PC (no immunomodulatory treatment), while there was a 20% to 25% reduction of the preoperative DTH score in PA (Indo). T-cell receptor (CD3+, CD4+, IL-2R+) protection appeared to be excellent in PB during the postoperative phase, with a tendency toward overcorrection for the CD4+ and IL-2R+ subpopulations on day 7, while simultaneously, under this respective regimen, the counterregulatory activity to limit excessive monocytosis seemed to be most effective. Although Indo administration could alleviate the quantitative unbalances of PBMC subpopulations seen in PC, it could not continuously induce significant improvement as demonstrated in a recent study[16] that investigated different types of operative trauma (gastrectomy, abdominal aortic grafting).

Postoperative depression of lymphocyte blastogenesis was also most sufficiently counteracted with combined TP-5/Indo therapy, which clearly worked better than the two-shot sole TP-5 administration, as demonstrated in a previous investigation in an identical operative model.[15] In that study with Indo, the impairment of proliferative responses was also significantly reduced.

In vitro IL-2 synthesis in cell cultures of PB on consecutive postoperative days did not show any suppression and the values were always the same or more than the baseline level. To our knowledge these findings represent the first report of a successful intervention to preserve adequate T-cell capacity for IL-2 production in humans after trauma.

The serum levels of γ-IFN in the patient population treated with Indo/TP-5 were continuously increasing on consecutive postoperative days, with an average increase of +50% above baseline between days 3 and 7. The increased amount of γ-IFN released in PB provides evidence for the complex efficacy of T-cell activation, triggered through that specific immunomodulatory treatment. Gramma interferon serum levels in PA were within the baseline range on day 7, in contrast to an average reduction of 35% compared to preoperative levels in PC on day 7.

D-erythro-Neopterin, an indicator of Mϕ activation,[23] increased more rapidly in PC within the first 72 hours after trauma compared to PB. By day 7 after operation, NPT concentrations were identical in all three patient groups.

In more recent subsequent tests within this investigation, we analyzed the supernatants of LPS-stimulated PBMC cultures of PA, PB, and PC for the proinflammatory cytokines IL-1, IL-6, and tumor necrosis factor (TNF) (results not shown here). The results showed that interleukin-1 (IL-1) synthesis in PB cultures was significantly higher compared to PA and PC. When including these findings into the body of information derived from this study, one can find striking evidence for the salutary mechanism of action induced by the Indo/TP-5 treatment.

Simultaneous PGE_2 blockade *via* Indo and T-cell activation *via* TP-5 apparently represent an ideal strategy to provide protection for the successful development of an immune response for which several levels of control are necessary. Essential steps within the forward-regulatory immune response pathway include: synthesis and release of IL-1 from Mϕ, intact Mϕ function, intact T-cell receptor function, a sufficient number of functionally intact T-helper cells, IL-2 synthesis and adequate IL-2 release, as well as IL-2–sensitive responder cells with intact capacity of IL-2 receptors.[24] Furthermore Mϕ participation can only be sustained *via* γ-IFN production by activated T cells. The downregulation of CMI responses occurs mainly due to PGE_2, a regulatory mediator released from inhibitory monocytes, which are released in large amounts after injury. One of the most prominent PGE_2 immunoregulatory activities consists of the regulation of IL-2 synthesis.[25] Mediators such as PGE_2, which are associated with both specific and nonspecific immunosuppression, have been demonstrated to impinge on the prostaglandin cyclic AMP pathway and it has been postulated that agents that interfere with these mechanisms may counter the immunosuppressive influences. Thus it has been recognized that an immunopharmacologic attack on T-cell disturbances in trauma should either contrasuppress the suppressor–PGE_2 by blocking cyclic AMP, or it should have immunorestorative capacities in terms of promoting the cyclic GMP, as for example, thymomimetic substances such as TP-5 do.

Based on these reflections it appears logical that a treatment regimen that combines blocking, ideally with enhancing action, should consist of a combination of immunopotentiating agents that complement each other functionally. Maghsudi et al.,[26] in an animal model of burn injury, demonstrated that the restoration of depressed secondary immune responses to sheep red blood cells could be best accomplished through the additive effect of Indo and TP-5.

Our results demonstrated that using the combination of TP-5 and Indo, all essential components within the lymphokine cascade circuit could be either protected or stabilized after ECC.

When interpreting these data, however, one must stay away from oversimplification, although a dissection of the mechanisms is warranted. It will always remain difficult to demonstrate the absolute causality between a particular agent and a seemingly corresponding function. Many of the findings in this study indeed represent the result of amplification achieved through directing the therapy on two targets, the Mϕ and the T cell.

While excessive PGE_2-dependent monocytosis, for example, could be counteracted with Indo, as shown in a previous study,[16] we noted in the present investigation that a considerable additive effect can be derived from the combination with TP-5. It appears that TP-5 probably is acting by blocking off some suppressor-active T-cell subpopulation.[27]

The preservation of functionally intact T-cell subpopulations (CD3+, CD4+) also clearly results from synergistic action of both agents, whereas undisturbed IL-2R expression appears to be mainly a function of cyclooxygenase inhibition.[28]

The successful preservation of IL-2 under stressful conditions, evidently, must be the consequence of the smooth interaction of several factors: the presence of adequate numbers of CD4+ cells, the induction of cyclic GMP and simultaneous shut off of cyclic AMP, as well as the presence of IL-1 synthesizing Mϕ, all of which are optimally conditioned *via* Indo/TP-5 support. Adequate production of γ-IFN from T cells depends on the presence of IL-2 in these cells. Most convincingly in this study, the preoperative serum levels of γ-IFN could only be sustained in those patients (PB) in whom the *in vitro* stimulation of PBMC cultures also resulted in intact IL-2 synthesis.

In a recent multicenter study with low-dose administration of γ-IFN to patients with multiple trauma, Polk and colleagues[29] showed the mechanistic impact of this mediator for the preservation of Mϕ forward–immunoregulatory capacity in terms of HLA-DR expression after injury.

In a most recent *in vivo* experimental investigation Ertel et al.[30] demonstrated the potential of maintaining Mϕ function following hemorrhagic shock, with cyclooxygenase inhibition. These investigators showed that the administration of the NSAID ibuprofen resulted in a significant improvement of the antigen presentation capacity, IL-1 and TNF/cachectin synthesis in peritoneal Mϕ.

In a further study using the identical shock model, these authors could find evidence that the administration of ibuprofen following hemorrhage resulted in maintenance of IL-2 and γ-IFN synthesis in splenic lymphocytes as well as of IL-1 in splenic Mϕ from C3H/HeN mice[31] following hemorrhage. In contrast untreated hemorrhaged mice showed massive depression of these respective functions.[31]

The concept of strengthening the facilitory component—while simultaneously suppressing the downregulatory PGE_2-mediated component—of Mϕ behavior has also been advocated by Browder and coworkers.[32] In a randomized, prospective, double-blind study they investigated the effect of glucan, a macrophage stimulant, on CMI function in patients with multiple injury and found that glucan treatment resulted in a rapid increase of IL-1 in serum, which was also correlated with subsequent skin test conversion of anergic patients to positive. While the use of such an immunopotentiating agent may represent an intriguing approach to immune response modification, the administration of cyclooxygenase inhibitors, however, is intended to go far beyond stimulation in its therapeutic impact. Nonsteroidal anti-inflamatory drugs, by breaking down the inflammatory cycle, by limiting the nondiscriminant whole-body hyperinflammation, represents in our experience the key step to avoid the overstimulation of the Mϕ during the initial post-traumatic phase. A number of agents, some of them acting in a self-sustaining, autoregulatory fashion, such as PGE_2, and IL-6 complement split products or lipopolysaccharides, it seems, cannot turn on the deleterious suppressor mode in the Mϕ after trauma, when the increase of cyclic AMP is counteracted with a NSAID. In this study we could show that the combination of Indo and TP-5 could alleviate the steep increment of the Mϕ metabolite NPT as an indicator of a Mϕ-driven hyperimmune response[24] compared to PC. We postulate that for the intact CMI function, the potentially deleterious acute-phase reaction can be downregulated with Indo/TP-5. This suggestion also has been corroborated by our data (unpublished), which showed a significantly lower IL-6 release in LPS-stimulated PBMC cultures compared to PC.

The analysis of postoperative infectious complications within the patient population studied demonstrated a total of three cases with severe septic episodes with gram-negative bacteria in PA/PC and one case of sternal osteitis through staphylococcus epidermidis. Two of the three patients with sepsis died. In PB there were three cases of minor infectious episodes. Obviously the patient numbers within that specific operative model are too small to allow conclusions in regard to clinical relevance.

This study quantified and specified with a tridimensional set of parameters the massive impact of open heart surgery under ECC on CMI.[33] Scrutiny of the efficacy of different immunopotentiating substances revealed that combined therapy of Indo and TP-5 is superior to sole Indo administration. Indo/TP-5 therapy resulted in a most adequate protection of all T-cell functions tested and it alleviated postoperative monocytosis and $M\phi$ overactivation. We found a convincing concurrence between the results of our long-term series of *in vitro* mechanistic studies and the findings derived from the simultaneous *in vivo* administration of blocking, and enhancing, agents in terms of protection of adequate $M\phi$/T-cell interaction following major operative trauma.

Acknowledgments

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DISCUSSION

DR. ARTHUR E. BAUE (St. Louis, Missouri): This is the first demonstration of the ability to restore immune competence as we presently know it, and to define it in a group of patients after operation, and after that type of injury.

The title that Dr. Faist used is "Immunmodulation," but I think actually it is "immunostimulation." The thymopentin is stimulatory and the cyclooxygenase inhibitor allows the full expression of interleukin-2 (IL-2) and gamma interferon. He also has demonstrated that it is safe to use these drugs in this group of patients.

It was the poet Gertrude Stein who once said that a difference in order to be a difference must make a difference. The question is then, in this group of patients, did it make a difference?

I had the opportunity to review Dr. Faist's manuscript before the meeting, and he does provide the clinical results. Perhaps he can comment further about this in his concluding remarks. He found that there were only three very minor or trivial infections in the group that received indomethacin and thymopentin, in contrast to several severe infections and a death in each of the other groups. He points out in the manuscript that this is too small a group of patients to make any deductions, but that this therapy does look hopeful initially. He needs more numbers for this, and I am sure they will be forthcoming.

This group of patients, in spite of the abnormalities of cardiopulmonary bypass, has a predicted low incidence of infection. You would not expect them to have many difficulties. This study should now be carried out in a higher risk group. Multiple-trauma patients with a higher risk to develop infection would be appropriate.

My questions for Dr. Faist are: (1) Will it be safe to use indomethacin and thymopentin in trauma patients? (2) Will it interfere with hemostasis? (3) Will there be problems with platelets in the multiple-system–injured patient? (4) Did he note any hemostatic differences in this group of patients, or was it similar to the results with aspirin? Was there more blood loss in those receiving indomethacin?

Finally why is there immunosuppression after injury? Is there some biologic method in this madness that is yet to be uncovered?

If there is can we fool Mother Nature and alter this response?

DR. HIRAM C. POLK, JR. (Louisville, Kentucky): Ladies and gentlemen, these papers, I think, crystallize some ideas that have been developing for most of the last decade. First of all I do think the critical role of the circulating monocyte and its capacity to express and present class 2 histocompatibility antigen is important as a major factor in therapy.

I think for many working in this field as clinicians, it is hard to understand the new words, and I think it has helped us most to realize that all of these mediators, toxins, kinins, and cytokines have the capacity to be harmful at one point and to be, in another dose in another time, perhaps helpful. All of those can interfere and, to a degree, although they do interfere at one time and place, they also can promote recovery.

I think it is very much like endotoxin, as a prototype; multiple small doses are profoundly protective, and large doses are indeed destructive and even fatal.

The ebb and flow of IL-1 or IL-infinity, or whatever you want to study, is such that, to some degree or another, they will predict what will happen. I suspect that in the not distant future, just as we use pulse oximetry today, there will be a method to monitor these substances from moment to moment, day to day, and to alter therapy accordingly.

I think this will be practical, and I think it is going to be essential to answer some of the questions that have been brought up in discussion of both of these papers.

Just as Drs. Meakins and Carrico pointed out previously, the use of this pattern of either graded, combination, or sequential therapy, treatment that may or may not be combined, is just the same kind of thing we do today with antibiotic sensitivities and resistances. We are looking for the right drug, the right route, and the right time.

Dr. Faist's paper is an important one because, as Dr. Baue pointed out, with the small groups and the low attack rate, it could not conceivably have answered the ultimate question, and that is, does it make a difference in clinical outcome? Conversely in reading the manuscript I think Dr. Faist has brought together several points: his own data, many other published animal data, and now human data suggest that you can alter some of these physiologically valid parameters sequentially in a fashion that is protective to the patient.

The further giant step that is going to be required is showing that this changes clinical outcome with somebody going home from the hospital who otherwise might not have done so. Clearly, that could not have been answered with this trial, but he surely has set the stage by suggesting that multiple therapy, sequential therapy, and combination therapy may make a difference to this ultimate end.

DR. JOHN R. BORDER (Buffalo, New York): These two papers by Grbic et al and Faist et al are of critical importance. Surgery is nothing more than the management of wounds and their systemic consequences. Wound biology is a product of cell biology. The essence of the wound cell biology is the activation of the leukocytes as a system with output of cytokines, eicosanoic acid derivatives, and leukocyte destructive products. This system involves coordinated activities between the polymorphonuclear cells, the monocyte-macrophage, the various lymphocytes, the endothelium, and certain tissue cells such as the MAST and probably the eosinophil.

The essential point is that this is an organized response that systemically, through the septic response, mobilizes the oxygen, energetic, and synthetic substrate required. Locally it mobilizes the cells required, and releases the materials required to prepare devitalized tissue for phagocytosis, phagocytizes this prepared material, and then digests it. The devitalized tissue is always self-devitalized tissue so that strong immunosuppression occurs in the wound. Prostaglandin E_2 is one of the immunosuppressant agents. Because the leukocyte destructive agents required for removal of devitalized tissue can clearly damage normal tissue if they escape, there is yet another component to the problem. Simultaneous with the release of wound agents (cytokines, eicosanoic acid derivatives) that produce the septic response, there is also activation of the acute phase system. This contains many elements that are capable of neutralizing the leukocyte destructive products.

The preceding system is activated normally by and in proportion to the devitalized tissue in the wound. This activation is proportional to wound size in elective surgery where there is minimal devitalized tissue and is required for normal wound healing.

We must understand two consequences of this system. In the local wound, the cellular response and the release of leukocyte destructive products expands the local wound by converting marginally viable tissue to devitalized tissue. Therefore there are two local wound sources of devitalized tissue. The first is from the initial wounding agent and the second is from the leukocyte system response to the devitalized tissue produced by the initial trauma. This point is of considerable importance because there are a variety of ways the second tissue insult can be manipulated when the biology of the wound is properly understood. Ward has shown for burn wounds that, if after the flame is applied a xanthine oxidase inhibitor that also blocks release of mast cell histamine is applied, a burn wound does not evolve. This same principle also applies to the gut wound. The principle of an immediate and then delayed tissue devitalization has to be a general one that allows new opportunities for therapy.

The problem arises in major trauma of all kinds that we have, in addition to the known wound activation of this system, the development of a gut wound that causes activation of the same system by the damage done to the gut mucosa and by translocation of endotoxin and bacteria. Thus in major trauma there are always two wounds that produce activation of the leukocyte system. Unfortunately in the gut wound there is much less of a diffusion barrier for the leukocyte destructive products. Those destructive products then have much more access to circulating blood and therefore to the liver and lung. Adult respiratory distress syndrome (ARDS) means both that these destructive products have damaged the lung and just as importantly have activated the pulmonary leukocyte system so that there is now a pulmonary wound. Successful therapy requires that we treat the primary wounds properly, treat the gut to prevent the gut wound, and thereby prevent the pulmonary wound.

The discussion has been presented this way because basic biology has now progressed to the point where it may become directly useful therapeutically. This is well illustrated in these two papers for one small portion of the concepts employed. There are many others. Later Dr. Wilmore will speak about glutamine-enriched solutions preventing septic complications. That paper must be viewed as reducing the magnitude of the gut wound. We also may use therapy to directly neutralize leukocyte destructive products. These two papers of Grbic et al and Faist et al show that we can begin to influence the systemic consequences of the various wounds once we understand the pathophysiology well enough. That is not the same as saying the influence will be beneficial, but it is a start. Basic biology is clearly presenting us with many new therapeutic possibilities.

DR. FRANCIS D. MOORE (Boston, Massachusetts): Some years ago before this society, Dr. Altemeier discussed the immunosuppressant effects of antibiotics. This was an important problem and still is. Some of them are immunosuppressive because of molecular configuration and their pharmacologic effect on marrow or on leukocyte function; others by random metabolic alteration, often unpredicted, in the microflora of the patient so as to diminish or abolish antigenic stimulation of the patient.

I just wonder if any of these studies have been done in the absence of antibiotics. Probably not. Possibly open heart surgery. I do not know. Antibiotic effects are widespread. And secondly I wonder if anybody has studied some normal volunteers, given them antibiotic mixes of the type used here, to find out if at 7 to 14 days they are not showing some of these same immunosuppressive effects.

DR. DONALD E. FRY (Albuquerque, New Mexico): I would like to address a couple of questions in this very nicely presented paper. Number 1 is the specificity of the treatments that were employed; and number 2 is the adequacy of the controls to make all of the sweeping conclusions that have potentially been made.

In our experiments using indomethacin in septic animals, it has a marvelous short-term improvement in survival, but does so only at the price of having decreased long-term survival in animals because of its renal consequences. Indomethacin as a comprehensive suppressant of cyclooxygenase may well mean that although it may stimulate lymphocytes, as the authors have addressed, it may extract a significant price from renal function and kidney perfusion.

So I would like first to ask Dr. Faist whether they have studied the creatinine, perhaps even had studies of glomeruli filtration and so forth in any of their treatment models with indomethacin, because I think there is generally a perception that comprehensive cyclooxygenase inhibition may have more effects than simply specifically enhancing lymphocyte function as he has illustrated.

Secondly it is always nice to talk about inhibition of cyclic adenosine monophosphate (AMP) and stimulation of cyclic guanosine monophosphate (GMP) and get into the molecular biology frenzy that seems to be permeating the literature right now, but conversely, the question is, have these experiments proven that? There is a critical control failure in that there is not a "Tp-5 only" treated group of individuals. It may be that the indomethacin as being used in these experiments actually is totally unnecessary to get the observations that he has made, in that he does not have a pure Tp-5 group.

So I would be interested to know, in other experiments, has he seen whether indomethacin really adds anything to the Tp-5 benefit?

DR. PISSIOTIS (Athens, Georgia): I think Dr. Faist should be congratulated on a fine, well-designed and conducted study. It was, however, done in cardiac surgery patients, who rarely get infections. Before we engage in this "wide world" of immune enhancement prophylactically in all patients, I think maybe Dr. Faist and others should show that immune enhancement is really effective in high-risk patients or in patients that are already infected, or both.

This is a dangerous and costly therapeutic modality, and before it is applied clinically, it should be applied prophylactically in high-risk patients and therapeutically only in patients who have already developed an infection or who are immunocompromised.

This is the first clinical study in this field, however, and Dr. Faist must be commended.

DR. E. FAIST (Closing discussion): I would like to thank all the discussants for their kind comments and questions.

Dr. Baue expressed his feeling that this study was dealing with immunostimulation rather than with immunomodulation. This might partly be true, but in general I would state that the effect of cyclooxygenase inhibition goes far beyond immunostimulation with any other pharmacologic agents, as besides modifying cell-mediated immune responses, nonsteroidal anti-inflammatory drugs (NSAIDs) counteract the hyperinflammatory response, especially the nondiscriminant hyperinflammation after overwhelming trauma. Thus besides targetting on the immunomechanistic negative effects of PGE_2, cyclooxygenase inhibition also is supposed to alleviate hyperinflammation. It can shut off the inflammatory cascade at a time when we need still to interfere, so as not to upregulate the release of proinflammatory cytokines, which, when present in excessive concentrations, further promote immunosuppression.

Multiple trauma probably was not foreseen during the evolutionary process, nor was—which is much more important—the initial survival after major injury through the modern means of resuscitation. The late septic complications that we see today in post-traumatic courses are a consequence of successful modern medicine. Still 10 to 15 years ago, I would say, many of these patients in whom we now state severe immunodepression, which appears sometimes, to be hardly reversible, have not survived their injuries. Thus we deal with a phenomenon of medical progress. This might not be the whole answer, but I think under this aspect we have to discuss the whole issue.

In answer to Dr. Fry's questions, I would like to state: Certainly we had an adequate control group. The quality and quantity of operative procedures was identical in all three patient groups.

Further on also answering to Dr. Pissiotis: We have to distinguish if one uses cyclooxygenase inhibition in a perioperative—or in an interventive—intention, *ergo*, at a time when infection has already occurred. This represents a most crucial difference, and is most decisive for the success of a such an intervention! We use cyclooxygenase inhibition to counteract the whole massive circuit of inflammation, which sets the stage for all ongoing downregulation of cell-mediated immunity.

As outlined in our manuscript, Dr. Fry, a previous pilot study with TP-5 in patients undergoing cardiac surgery had been carried out. There we have found that single-agent TP-5 administration is very well potent to restore respectively preserve delayed-type hypersensitivity compared with preoperating baseline values, and also can augment lymphoblastogenesis, but it did not show any influence on receptor protection and, most importantly, it cannot preserve adequate interleukin-2 synthesis.

The administration of indomethacin in this protocol did not result in any side effects. We now oversee several complete series of studies, not only in cardiac surgery, but also in oncologic patients undergoing general surgery, comprising a total of 200 patients, and under our regimen of a maximum of 150 mg indomethacin per day, we did not observe any problems in terms of renal toxicity, gastrointestinal bleeding, or thrombocyte disorders.

I agree with Dr. Fry that the effects of indomethacin go far beyond immunostimulatory properties, as it has many more beneficial effects in the stressed individual, like the attenuation of hypermetabolism or the influence on the brain–immune axis.

Again I would like to thank all discussants for their kind remarks and especially the ASA for the honor of letting me present our work at this impressive forum.

Chronic Visceral Ischemia

Three Decades of Progress

CHRISTOPHER G. CUNNINGHAM, M.D., LINDA M. REILLY, M.D., JOSEPH H. RAPP, M.D.,
PETER A. SCHNEIDER, M.D., and RONALD J. STONEY, M.D.

From the Section of Vascular Surgery, University of California, San Francisco, San Francisco, California

Symptomatic visceral atherosclerosis is a major surgical challenge because of its life-threatening course and the complexity of its definitive operative treatment. Evolution in the operative approach to the visceral aorta and progress in the intraoperative management of patients undergoing complex vascular reconstructions prompted a review of the authors' cumulative experience in the surgical management of chronic visceral ischemia. Among all patients undergoing visceral revascularization at the University of California, San Francisco during the past three decades, 74 patients were identified whose primary reconstruction used transaortic endarterectomy (TA TEA) (n = 48) or antegrade bypass (AB) (n = 26), the authors' preferred revascularization techniques. The two treatment groups were comparable in gender distribution, age, presenting symptoms, and physical findings, although the amount of preoperative weight loss was greater in the AB group (35.8 ± 19.5 *versus* 22.4 ± 12.0, p = 0.003). The groups were also comparable in the prevalence of atherosclerosis risk factors, symptomatic vascular disease at other sites, and previous vascular operations. However associated renal artery atherosclerosis was slightly greater in the TA TEA group (58.3% *versus* 23.1%, p = 0.07) when compared to the AB group. Antegrade bypass was usually performed transabdominally (88.5%), while TA TEA was approached thoracoretroperitoneally (75.0%). Celiac revascularization was almost universal in both treatment groups, but the TA TEA group underwent significantly more frequent superior mesenteric artery (SMA) revascularization (93.8% *versus* 46.2%, p = 0.0001) and slightly more frequent inferior mesenteric repair (18.8% *versus* 3.8%, p = 0.07) than the AB group. In addition the frequency of combined renal and visceral repair (25.0% *versus* 0.0%, p = 0.01) as well as combined aortic, renal, and visceral repair (22.9% *versus* 3.8%, p = 0.03) was significantly greater in the TA TEA group. The obligatory interval of renal and visceral ischemia did not differ between the two approaches. The perioperative mortality rate was 12.2% and was the same for TA TEA (14.6%) and AB (7.7%). Overall the incidence of complications was the same with either operative approach, although patients in the TA TEA group tended to have multiple complications (17.1% *versus* 0.0, p = 0.03) and all significant pulmonary complications occurred in this group. Two patients were lost to follow-up. The cumulative percentage of patients who remained asymptomatic following AB or TA TEA was (respectively) 95.8% and 97.3% at 1 year and 86.5% and 86.1% at 5 years. Both of these operative approaches provide durable symptom relief with acceptable operative morbidity and mortality rates. These results are attributed to intraoperative transesophageal echocardiography, to intraoperative assessment of the visceral reconstruction with Duplex ultrasonography, and to the increasing use of the transabdominal approach, with medial visceral rotation, to provide aortic exposure for both AB and TA TEA techniques.

ATHEROSCLEROSIS IS the most common cause of obstruction of the orifices of the celiac and mesenteric (visceral) branches of the aorta.[1–4] The resulting reduction in splanchnic blood flow produces clinical symptomatology, which typically begins as postprandial ischemic pain, called 'intestinal angina' by Mikklesen.[5] Chronic, symptomatic visceral ischemia is a progressive, nutritionally disabling precursor to fatal intestinal infarction, although the exact proportion of patients who will eventually develop this is unknown.[1,6–8] Surgeons pioneered the understanding of chronic visceral ischemia and introduced the first operations for revascularization of the visceral aortic branches more than three decades ago.[2,3,8–14]

Evolution in the operative approaches to the visceral segment of the abdominal aorta and progress in the intraoperative assessment and management of patients undergoing complex vascular reconstructions prompted us to review our 30-year cumulative experience in the surgical management of symptomatic chronic visceral ischemia.

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Address reprint requests to Linda M. Reilly, MD, Department of Surgery, Moffitt 488, University of California, San Francisco, San Francisco, CA 94143.

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This study investigates the safety and efficacy of visceral revascularization by transaortic endarterectomy or antegrade bypass, the two preferred techniques in our institution. We evaluated those adjunctive measures that might lessen the incidence of operative morbidity and improve the technical quality of the reconstructions, thereby contributing to long-term survival and durable relief of symptoms.

Methods

The records of all patients who underwent visceral revascularization at the University of California, San Francisco during the past the three decades were identified. The patients chosen for this study consisted of those who had chronic symptoms of intestinal ischemia and who underwent their primary visceral reconstruction at our institution. Specifically excluded from the study were those patients with acute visceral ischemia, those who had their initial visceral reconstruction at other institutions, those with median arcuate ligament syndrome, and those patients whose surgeon had performed less than ten visceral revascularizations at UCSF. The resulting study group of 85 patients (16 men and 69 women; mean age, 60.7 ± 10.5 years) consisted of 48 patients who underwent transaortic visceral endarterectomy (TEA), 26 patients who underwent antegrade visceral bypass, and 11 patients whose vascular reconstruction was performed using a variety of other techniques. The focus of the study was a comparison of the antegrade bypass and the transaortic endarterectomy treatment groups. Data were accumulated by review of inpatient, outpatient, and referring physician records. Follow-up data were accumulated by interview of the patients, their families, and the referring or primary care physician. Data were analyzed using the unpaired Student's *t* test, chi square contingency analysis and Fisher's exact test (one tailed), where appropriate. Follow-up data were analyzed in life table format, with the two specific endpoints being recurrence of intestinal ischemia symptoms (long-term relief of symptoms) and death from any cause (long-term survival). Life table curves were terminated when the standard error exceeded 10%.

Visceral Revascularization Techniques

Transaortic Endarterectomy

Initially transaortic endarterectomy of the visceral vessels was performed through a thoracoretroperitoneal approach (n = 36). Recently, however, we have obtained the same extensive aortic exposure through a transabdominal approach with medial rotation of the viscera (MVR) from the left (n = 7). In this technique, the left kidney remains in its anatomic position, while the plane behind the left colon, spleen, pancreas, and stomach is developed to allow displacement of these structures toward the midline (Fig. 1). The entire aorta, from the distal thoracic level inferiorly, can be completely exposed in this manner (Fig. 2). During complete aortic occlusion and with control of any intervening intercostal branches, a 'trap door' aortotomy is placed to circumscribe the orifices of the celiac and superior mesenteric arteries (SMA) (Fig. 3A) and an endarterectomy is performed, removing the aortic wall lesion and its extensions into the visceral orifices (Fig. 3B). In many cases in this series (n = 23, 47.9%), the significant aortic atheroma was confined to the ventral surface of the aorta immediately around the celiac and SMA orifices and the aortic endarterectomy was limited to the trap door segment itself. However more diffuse aortic disease, frequently in the setting of associated renal artery occlusive lesions, required extension of the aortotomy caudally to allow sleeve aortic endarterectomy and bilateral (n = 17) or unilateral (n = 6) renal artery TEA (Fig. 4). Following completion of the endarterectomy, the

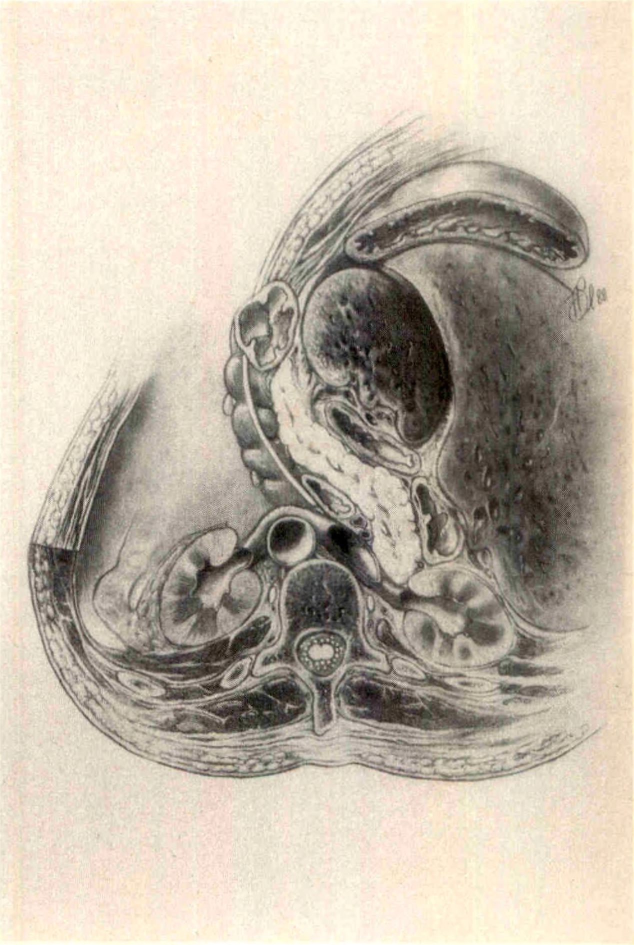


FIG. 1. Illustration of medial visceral rotation (MVR) approach to the aorta. Note that the kidney remains in its anatomic location, while the other abdominal viscera are displaced medially after mobilization from the left.

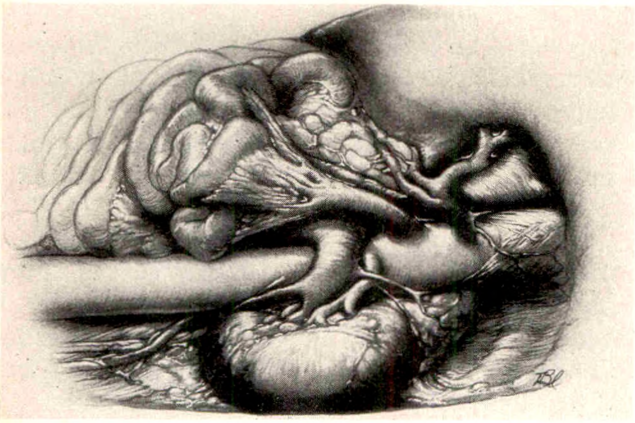
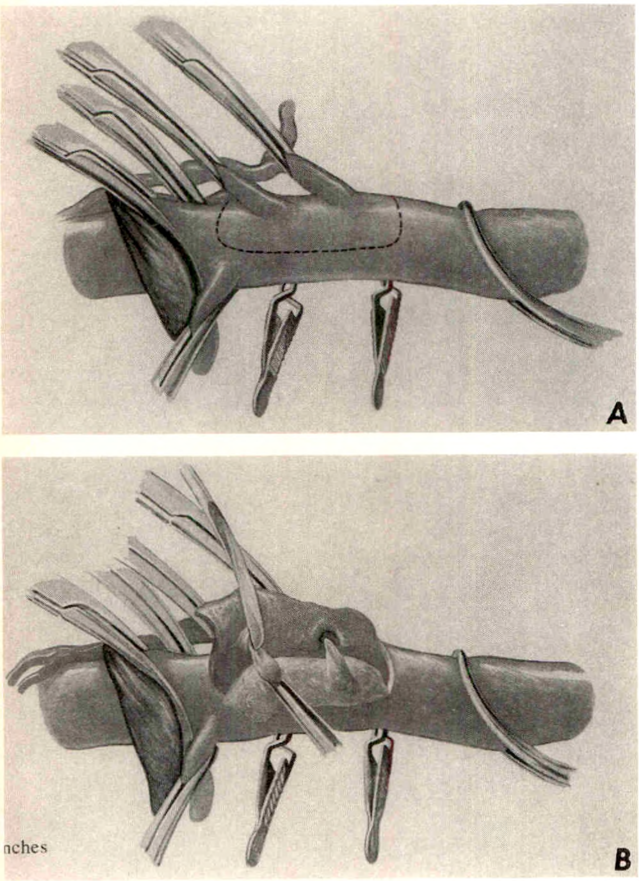


FIG. 2. The extent of the aorta that can be exposed easily after medial visceral rotation is shown. Note that the exposure is essentially the same as that provided by the thoracoretroperitoneal approach.

aortotomy is closed with a running suture (Fig. 5). In seven cases the planned orificial transaortic TEA could not be successfully completed due to transmural calcification or distal extension of the disease. In these cases the procedure was converted to a combination of transaortic TEA and other techniques, including antegrade bypass, interposition graft, transarterial TEA, and vein patch angioplasty.



FIGS. 3A and B. (A) Diagrammatic representation of trapdoor aortotomy circumscribing the visceral orifices. Note total proximal and distal aortic occlusion and control of individual intercostal arteries. (B) Trapdoor aortotomy has been opened and hinged on the right aortic wall. The endarterectomy is proceeding with removal of the SMA lesion.

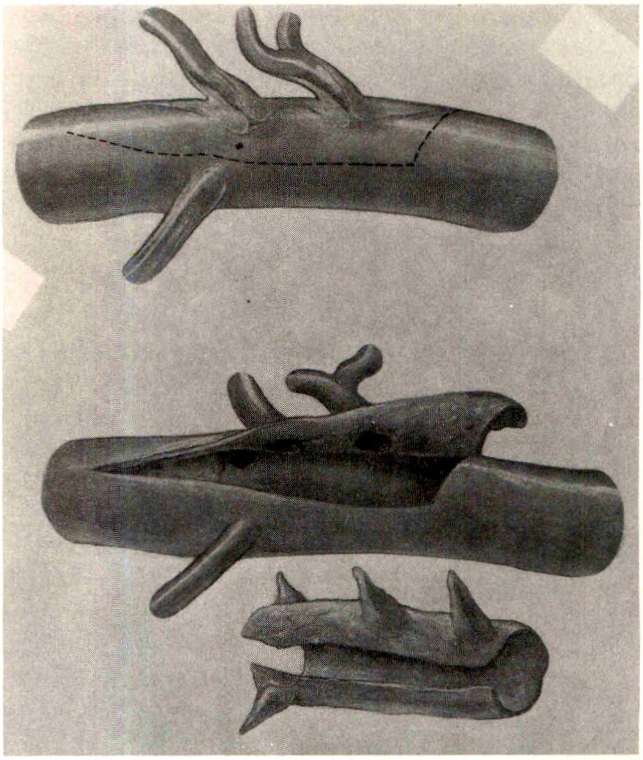


FIG. 4. Diagrammatic representation of the location of the modified trapdoor aortotomy used when associated renal artery disease requires combined visceral and renal endarterectomy.

Antegrade Visceral Bypass

Bypass grafts originating from the supraceliac, subdiaphragmatic aorta were initially performed using a transabdominal, transcrural approach to the aorta (n = 23), but medial visceral rotation has also been used in this patient group (n = 2). The proximal anastomosis, placed in the disease-free supraceliac aorta, requires temporary total aortic occlusion, However circumferential aortic mobilization is not routinely performed unless there is an intervening pair of intercostal arteries that need to be controlled. A small ellipse of aorta, oriented obliquely, is excised to facilitate the end-to-side anastomosis (Fig. 6). The celiac and SMA are exposed above and behind the pancreas, which requires careful and complete dissection from the celiac ganglion tissue and the median arcuate ligament. The distal anastomoses are constructed in an end-to-end manner to the individual visceral arteries, beyond the point of atherosclerotic involvement. The conduit is almost always prosthetic (n = 24, 92.3%).

Intraoperative Monitoring

Monitoring of cardiac function and assessment of the technical adequacy of the visceral repair have advanced

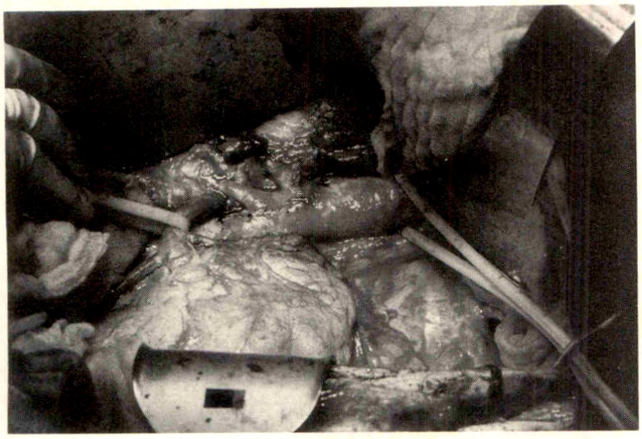


FIG. 5. Operative photograph showing the aorta and visceral vessels after completion of the endarterectomy and closure of the aortotomy.

with application of ultrasonographic imaging techniques. Two-dimensional transesophageal echocardiography allows real-time determination of cardiac filling status as well as detection of wall motion abnormalities[15] (Fig. 7). Duplex scanning of the vascular reconstruction provides immediate assessment of the appearance and flow characteristics in the bypassed or endarterectomized arteries.[16] These techniques have been used in 16 patients (21.6%) in this study group. In two cases (15.4%) significant technical defects were identified by Duplex scan that warranted reopening of the artery and allowed correction of the problem (Figs. 8 and 9).

Results

There was no difference between the antegrade bypass group and the transaortic TEA group in gender distribution, age at operation, presenting symptoms, or physical findings (Table 1). The majority of patients were women (61 of 74, or 82.4%) with classic postprandial pain (62 of 74, or 83.8%). Physical findings were generally nonspecific and most notable for significant weight loss, which averaged 25.7 ± 16.5 lbs for both groups, but was significantly greater in the antegrade bypass group (Table 1). The pattern of visceral vessel involvement did not differ between the two groups, with all but one patient having multivessel occlusive disease (Table 1). However the frequency of renal artery involvement by atherosclerosis was somewhat greater in the transaortic TEA group in comparison to the bypass group ($p = 0.07$, Fisher's exact test, one tailed).

The two treatment groups were also identical in the prevalence of risk factors for atherosclerosis, the prevalence of associated illnesses, and the frequency of previous abdominal and peripheral vascular surgery (Table 2). Although most patients smoked tobacco (55 of 74, or 74.3%), very few had diagnosed pulmonary disease (6 of 74, or 8.1%). Diabetes was equally uncommon. Symptomatic vascular disease affecting other vascular beds was common (44 of 74, or 59.5%), as was previous vascular surgery (29 of 74, or 39.2%). Notably four patients had already required segmental intestinal resection for ischemia or infarction some time before visceral reconstruction.

The transabdominal approach was used significantly more often for antegrade bypass, while the thoracoretroperitoneal approach predominated in the transaortic TEA group ($p = 0.0001$, chi square test) (Table 3). Recently both antegrade bypass and transaortic TEA have been performed using the transabdominal approach with medial rotation of the viscera from the left. To date MVR has been used equally often in both groups, although the numbers are still small. In both operative groups, celiac revascularization was almost universal, while the SMA was revascularized significantly less often among patients undergoing antegrade bypass than among those treated by transaortic TEA ($p = 0.0001$, chi square test) (Table 3). Inferior mesenteric artery reconstruction was also more frequent among transaortic TEA patients, but this did not quite achieve statistical significance ($p = 0.07$, Fisher's

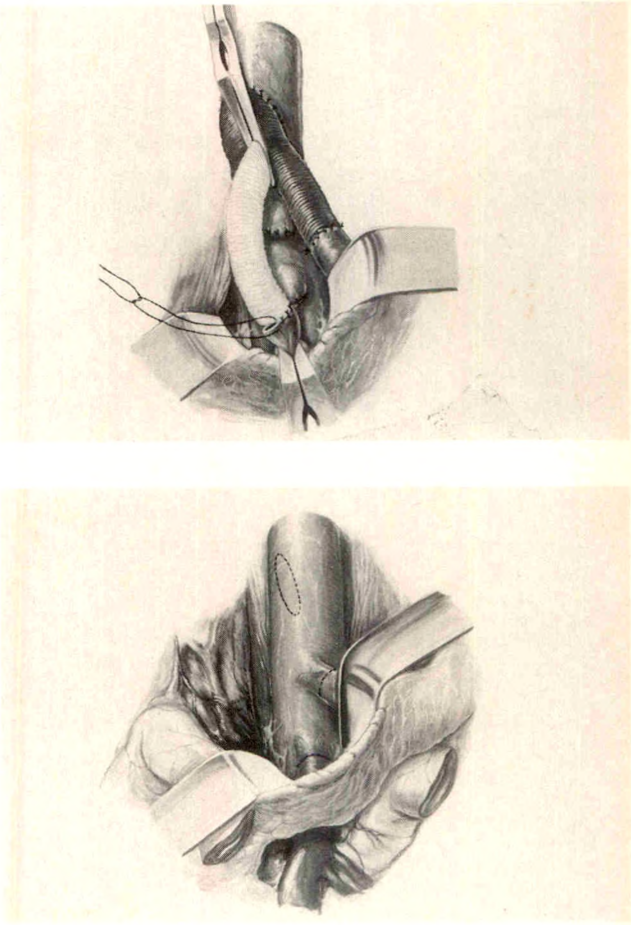


FIG. 6. Diagrammatic demonstration of the location and orientation of the proximal anastomosis for a bifurcated antegrade bypass to both the celiac and SMA. After completion of the proximal anastomosis, flow is restored through the aorta and the individual graft limbs are clamped for each visceral anastomosis.

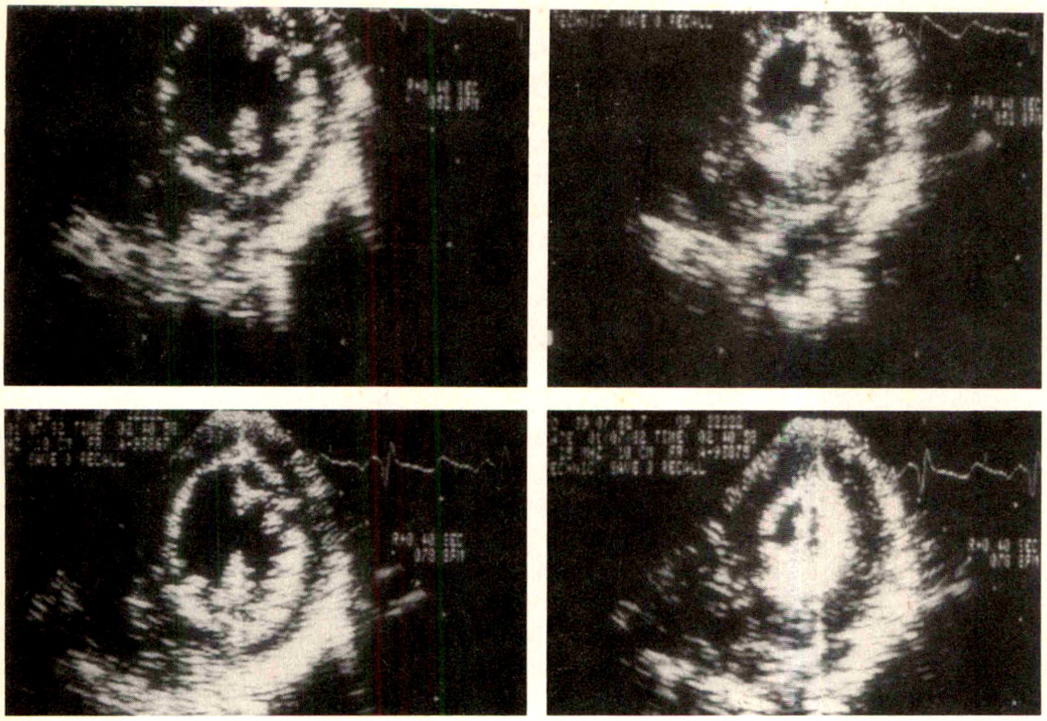
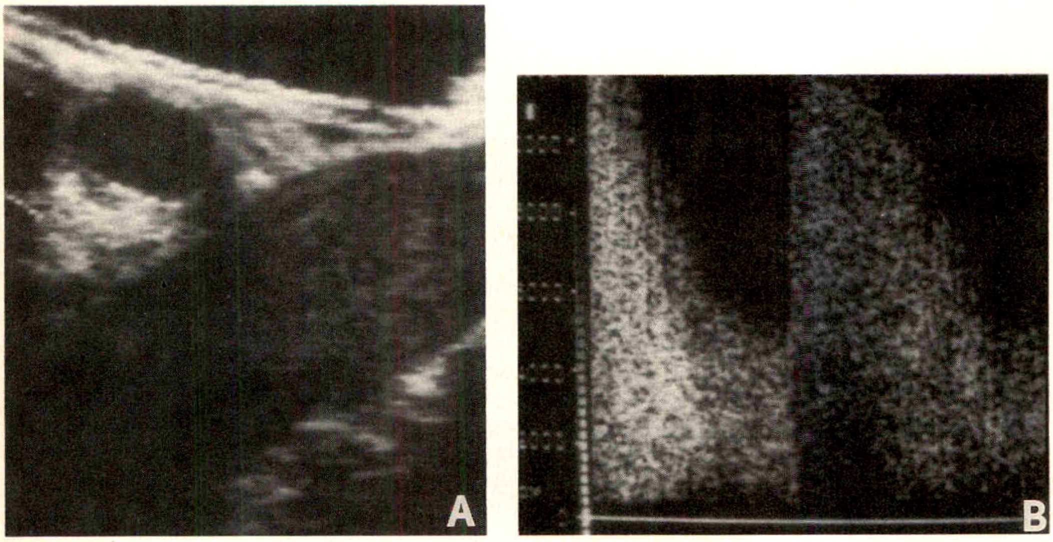


FIG. 7. Transesophageal echocardiographic views of the left ventricle, during systole (right frames) and diastole (left frames). Bottom frames show hypovolemia with empty left ventricle at end-systole.

exact test, one tailed). Overall the average number of visceral arteries repaired in the transaortic TEA group (2.10 ± 0.43) was significantly greater than the average number repaired in the antegrade bypass group (1.50 ± 0.58, $p < 0.0001$, unpaired Student's *t* test). As a consequence of their greater frequency of associated renal artery disease, transaortic TEA patients required concomitant renal artery reconstruction or concomitant aortorenal revascularization significantly more often than did antegrade bypass patients ($p = 0.01$ and $p = 0.03$, respectively, Fisher's exact test, one tailed). The obligatory period of visceral and renal ischemia resulting from total aortic occlusion did not vary between the two groups (Table 3).

Nine patients died in the perioperative period (12.2%), five from complications of bleeding, two of whom were re-explored for this bleeding. One patient developed pneumonia following a stroke and ultimately died of respiratory failure. One patient developed myocardial failure and died in multiple-system failure. One patient died of recurrent sepsis and enterocutaneous fistula formation following colonic perforation of uncertain etiology. The resected colon showed extensive mucosal ulceration but no transmural infarction and at autopsy, the visceral reconstruction (transaortic TEA) was patent without technical defect. One patient developed irreversible visceral ischemia intraoperatively in spite of a patent, functioning



FIGS. 8A and 8B. Intraoperative duplex scan of a celiac artery showing tight bandlike proximal stenosis (A) with elevated peak systolic frequencies (B).

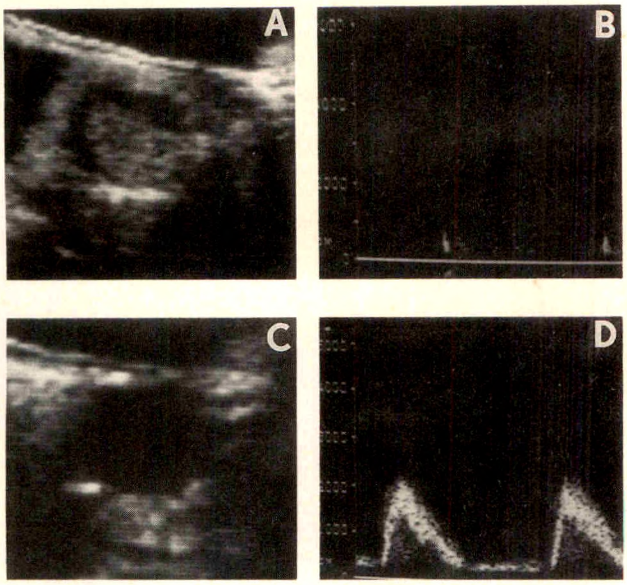


FIG. 9. Intraoperative duplex scan of superior mesenteric artery (SMA) after transaortic endarterectomy. (A) A large filling defect, with the appearance of thrombus, occupies most of the lumen and the associated flow signal (B) is almost nonexistent. (C) Appearance of the SMA following reopening to remove the thrombus and the associated intimal flap. (D) Note the normal flow signal in the artery after reopening.

repair (transaortic TEA). In spite of resection of the involved bowel (mainly colon), profound metabolic abnormalities led to her death intraoperatively. The exact mechanism of the development of the visceral ischemia was not established by examination of the resected bowel or at autopsy. Neither the mortality rate nor the overall complication rate was significantly different between the two operative groups ($p = 0.32$ and $p = 0.37$ respectively, Fisher's exact test, one-tailed), although both types of events occurred more frequently in the transaortic TEA group (Table 4). However the incidence of multiple complications was significantly greater in the transaortic TEA group ($p = 0.03$, Fisher's exact test, one-tailed), whether all complications or only those occurring in surviving patients were considered (Table 4). In addition, the type of complication varied significantly with the operative technique, with all of the pulmonary complications ($N = 11$) developing in the transaortic TEA group ($p = 0.04$, Fisher's exact test, one-tailed). Although all of the renal complications ($N = 3$) occurred in the transaortic TEA group, and all of the visceral complications ($N = 2$)—one graft thrombosis and one intestinal resection for infarction—occurred in the antegrade bypass group, the numbers were too small to detect any significance.

Two patients (2.7%) were lost to follow-up, both from the transaortic TEA group. The remaining 63 patients (85.1%) were followed for a mean interval of 71.1 months (range, 4.2–263.9). Nine patients (12.2%) developed recurrent symptoms of visceral ischemia. One patient (mentioned above) was known to have an occluded antegrade bypass (celiac and SMA) at the time of discharge. No further reconstruction was technically feasible. When his symptoms recurred as expected, resection of all ischemic and infarcted bowel was performed, but persistent inanition ultimately led to his death 14.1 months later. One other patient had persistence of all of her symptoms after transaortic TEA and arteriography showed occlusion of the SMA and stenosis of the celiac. About 5 months postoperatively, further visceral revascularization was attempted but could not be performed, and she died of extensive visceral infarction. The seven other patients experienced reappearance of their symptoms at 4.6, 14.6, 27.0, 28.9, 38.9, 66.3, and 98.1 months postoperatively, three following antegrade bypass and four after transaortic TEA. Three patients underwent a single repeat visceral revascularization. All again became asymptomatic and remain so 18.7, 108.6, and 179.4 months later. One patient was reoperated twice and then remained asymptomatic until the time of her death from a thoracic aortic dissection 55.8 months later. One patient was reoperated three times for recurrent visceral ischemia symptoms. After the final reconstruction, she had intermittent nausea and diarrhea, but gained weight and had no further deterioration of her status until her death 53.7 months later from complications after aortofemoral bypass grafting. The sixth patient had been troubled by repeated episodes of partial small bowel obstruction, which ultimately led to a perforation, complicated by recurrent enterocutaneous fistula formation. Three weeks after her fourth, and apparently successful, fistula resection, the patient returned to the hospital with acute mental status changes. She underwent exploratory laparotomy for a presumptive diagnosis of recurrent fistula formation with sepsis, but was found to have extensive bowel infarction and she subsequently died. The final patient who developed recurrent symptoms had a normal visceral angiogram. She subsequently underwent an exploratory laparotomy and resection of a short segment of ischemic bowel. She, too, remained asymptomatic for 59.4 months, when she died of a stroke.

The cumulative percentage of patients who remained asymptomatic following antegrade bypass and transaortic TEA was (respectively) 95.8% and 97.3% at 1 year, 87.8% and 86.1% at 5 years (Fig. 10). There was no difference in the long-term relief of visceral ischemia symptoms obtained with either of these two revascularization techniques. Similarly the long-term patient survival following either operation was equivalent: 92.3% and 80.6% at 1 year, 68.1% and 66.3% at 5 years, and 62.1% and 55.1% at 7 years for patients undergoing antegrade bypass and transaortic TEA, respectively (Fig. 11).

Only 27 patients (42.9%) underwent visceral angiography following reconstruction, one half before discharge (perioperative interval) and one half during later follow-up. The lack of follow-up arteriograms in a significant proportion of the study group prevents us from docu-

TABLE 1. *Clinical Presentation*

	Antegrade Bypass (n = 26)		Transaortic Endarterectomy (n = 48)	
	n	%	n	%
Age*	61.8 ± 12.8		59.8 ± 9.2	
Gender				
Women	20	76.9	41	85.4
Men	6	23.1	7	14.6
Symptoms				
Abdominal pain				
Postprandial	24	92.3	38	79.2
Atypical	2	7.7	10	20.8
None	0	0.0	0	0.0
Other gastrointestinal symptoms	14	53.8	33	68.8
Physical Findings				
Abdominal bruit	16	61.5	35	72.9
Positive stool guaiac	3	11.5	3	6.3
Abdominal peripheral pulses	16	61.5	24	50.0
Weight loss	23	88.5	36	75.0
Average (lbs)*†	35.9 ± 19.5		22.4 ± 12.0	
None	2	7.7	7	14.6
Preoperative weight (lbs)*	119.0 ± 20.6		116.3 ± 24.7	
Preoperative Aortography				
Visceral arteries involved				
Celiac	0	0.0	0	0.0
SMA	0	0.0	1	2.1
Celiac/SMA	10	38.5	16	33.3
Celiac/SMA/IMA	16	61.5	31	64.6
Renal arteries involved‡	6	23.1	28	58.3

* mean ± SD.

† p = 0.003 (unpaired Student's *t* test).

‡ p = 0.07 (Fisher's exact test, one-tailed).

SMA, superior mesenteric artery; IMA, inferior mesenteric artery.

menting the long-term patency of these visceral repairs. However, of 18 angiograms obtained in asymptomatic patients, 14 (77.7%) showed that all operated visceral vessels were widely patent (Fig. 12), 2 (11.1%) showed occlusion of a single visceral artery (celiac), and the results of two studies were not available.

Discussion

The surgical treatment of chronic visceral ischemia caused by atherosclerosis was initiated by the reports of Shaw and Maynard in 1958[9] and Mikkelsen and Zaro in 1959.[10] Both surgeons used transarterial TEA to reconstruct the SMA, which Mikkelsen believed was technically easier and more reliable than reimplantation or short bypass grafts. Despite this early experience, retrograde bypass grafting rapidly became the most frequently used technique for visceral revascularization.[3,7,8–11,14,17–24] This approach was preferred because exposure of the distal SMA and celiac branches for the distal anastomosis is easy,[3,14,22] infrarenal aortic exposure and control—the site of origin of these grafts—is easier than paravisceral aortic exposure and control,[3,11,14] there is no danger of dissection from the anastomosis,[8,11,14] and antegrade bypass can be performed from the transabdominal approach.[23] In addition some surgeons felt that visceral endarterectomy increased the difficulty of simultaneous aortic repair.[23] The consensus was that direct repair of occlusive celiac and SMA lesions was dangerous, unnecessary, and technically ambitious.[3,8,11] Williams[7] correctly noted that this early experience was almost uniformly lacking in any significant follow-up data and concluded that it had not actually been

TABLE 2. *Patient Profile*

	Antegrade Bypass (n = 26)		Transaortic Endarterectomy (n = 48)	
	n	%	n	%
Risk Factors				
Diabetes	1	3.8	1	2.1
Smoking	16	61.5	39	81.3
Hypertension	15	57.7	28	58.3
Hypercholesterolemia	3	11.5	10	20.8
Associated Illnesses				
Peripheral vascular	15	57.7	29	60.4
Cardiac	11	42.3	15	31.3
Renal	4	15.4	14	29.2
Pulmonary	3	11.5	3	6.3
Prior Surgery				
Peripheral vascular	12	46.2	17	35.4
Gastrointestinal tract	9	34.6	23	47.9
Gastrointestinal tract for ischemia/ infarction	1	3.8	3	6.3
Other	10	38.5	17	35.4

TABLE 3. *Operative Details*

	Antegrade Bypass (n = 26)		Transaortic Endarterectomy (n = 48)	
	n	%	n	%
Approach				
Transabdominal†	23	88.5	5	10.4
Thoracoretroperitoneal†	1	3.8	36	75.0
Medial visceral rotation	2	7.7	7	14.6
Arteries repaired				
Celiac	26	100.0	47	97.9
SMA†	12	46.2	45	93.8
IMA‡	1	3.8	9	18.8
Mean repaired arteries§	1.50 ± 0.58		2.10 ± 0.43	
Associated vascular repairs				
Visceral + aorta	2	7.7	2	4.2
Visceral + renal‖	0	0.0	12	25.0
Visceral + renal + aorta¶	1	3.8	11	22.9
Ischemia times				
Visceral*	26.0 ± 15.8		30.5 ± 15.5	
Renal*	25.2 ± 16.8		30.4 ± 16.0	

* mean ± SD.
† $p = 0.0001$ (chi square); ‡ $p = 0.07$; § $p < 0.0001$ (unpaired Student's *t* test); ‖ $p = 0.01$; ¶ $p = 0.03$ (Fisher's exact test (one-tailed).
SMA, superior mesenteric artery; IMA, inferior mesenteric artery.

established whether these visceral revascularization techniques would protect against fatal acute mesenteric infarction in the long term.

At about this time we became concerned that the limited exposure of transarterial TEA did not allow complete removal of the aortic portion of the atheromatous lesion and that reimplantation or retrograde bypass was threatened by the inevitable progression of atherosclerosis in the infrarenal aorta. Furthermore proper graft alignment was difficult with the retrograde grafts[25] and this prompted many surgeons to favor prosthetic conduits because they resisted kinking better[21-23] and some surgeons to modify the technique.[25,26] These concerns led us to adopt the almost exclusive use of transaortic endarterectomy or antegrade bypass from the supraceliac aorta as our two preferred methods of visceral revascularization. We have previously reported the initial and intermediate follow-up results obtained with these two techniques.[27,28] Accumulating experience and continuing observation of the patients now allows us to present long-term follow-up data in life table format and to compare the results obtained in the two operative treatment groups.

TABLE 4. *Perioperative Results*

	Antegrade Bypass (n = 26)		Transaortic Endarterectomy (n = 48)	
	n	%	n	%
Outcome				
Alive	24	92.3	41	85.4
Dead	2	7.7	7	14.6
Complications (all patients)				
None	16	61.5	23	47.9
One	8	30.8	12	25.0
Two or more*	1	3.8	11	22.9
Complications (surviving patients)				
None	16	61.5	23	47.9
One	8	30.8	11	22.9
Two or more*	0	0.0	7	14.6

* $p = 0.03$ (Fisher's exact test, one-tailed).

Transaortic Endarterectomy

It has long been recognized that obstructing visceral atherosclerotic lesions are usually confined to the first 2 cm of the vessel origins. Initially it was not appreciated that these lesions really represent an overflow of aortic atherosclerosis into the visceral vessel orifices. Thus, although the limited extent of the lesion in the visceral artery makes transarterial endarterectomy attractive, complete removal of the aortic component of the atheroma requires a transaortic approach. We had found this to be a successful technique for renal artery occlusive disease and felt it was applicable to the visceral vessels as well. However safe performance demands unrestricted access to the upper abdominal aorta. The early transaortic visceral TEA procedures in this series were performed transabdominally, from the front of the aorta (n = 6). This resulted in very limited exposure, and complete removal of the visceral portion of the lesion was difficult, and often associated with tearing of the aorta or visceral vessel. Subsequently we began to use a left thoracoretroperitoneal approach,[29] which was a modification of previously reported retroperitoneal and thoracoabdominal exposures of the aorta.[30,31] The realization that medial rotation of the viscera from the left provided the same extensive aortic exposure[32] through the transabdominal approach now allows us to avoid entirely the use of two body cavity pro-

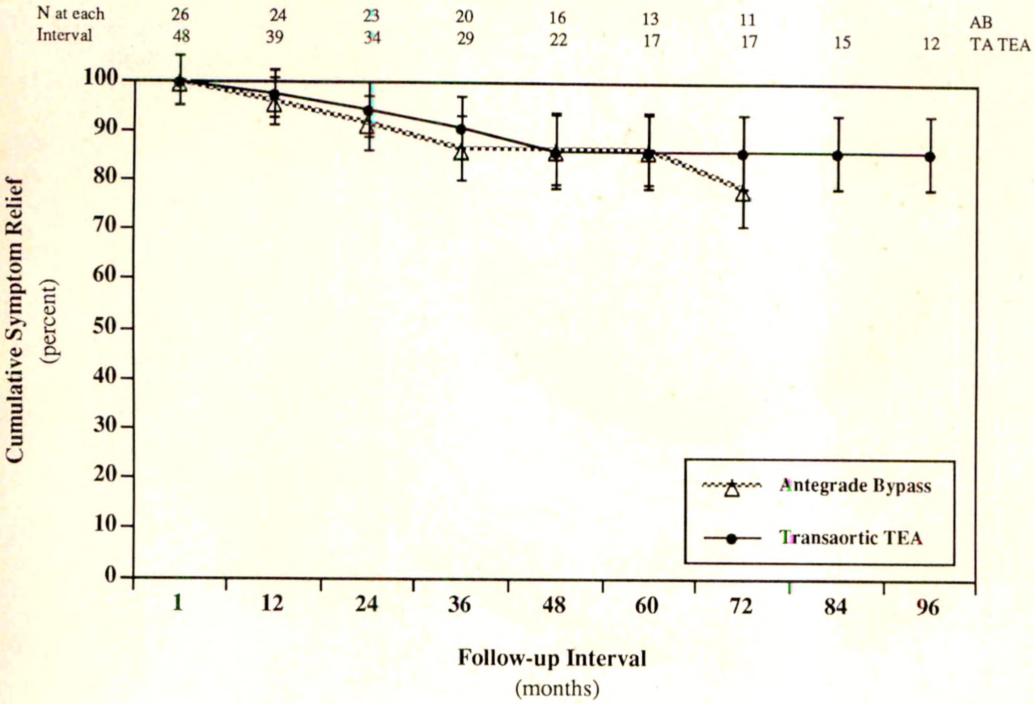


FIG. 10. Life table representation of the cumulative symptom relief provided by antegrade bypass (AB) and transaortic endarterectomy (TA TEA). All SE less than 10%.

cedures. Because all of the substantial pulmonary morbidity in this series occurred following transaortic TEA, we think that this modification in approach represents a significant improvement that will increase the applicability and safety of the transaortic TEA technique. Inevitably there will be some arteries that will not be amenable to endarterectomy, as indicated by the seven times we had to modify this technique in our patient group. The most common reasons for this are transmural calcification and very distal involvement of the visceral vessels by the occlusive process. It is imperative that the surgeon recognize this early and switch to an alternative approach before prolonged visceral ischemia has occurred. Visceral ischemia invervals exceeding 1 hour are almost always fatal. We did not find that transaortic TEA made concomitant aortic repair difficult,[23,33] as evidenced by the fact that 27.1% of the patients in the operative group (n = 13) underwent simultaneous aortic reconstruction. Tran-

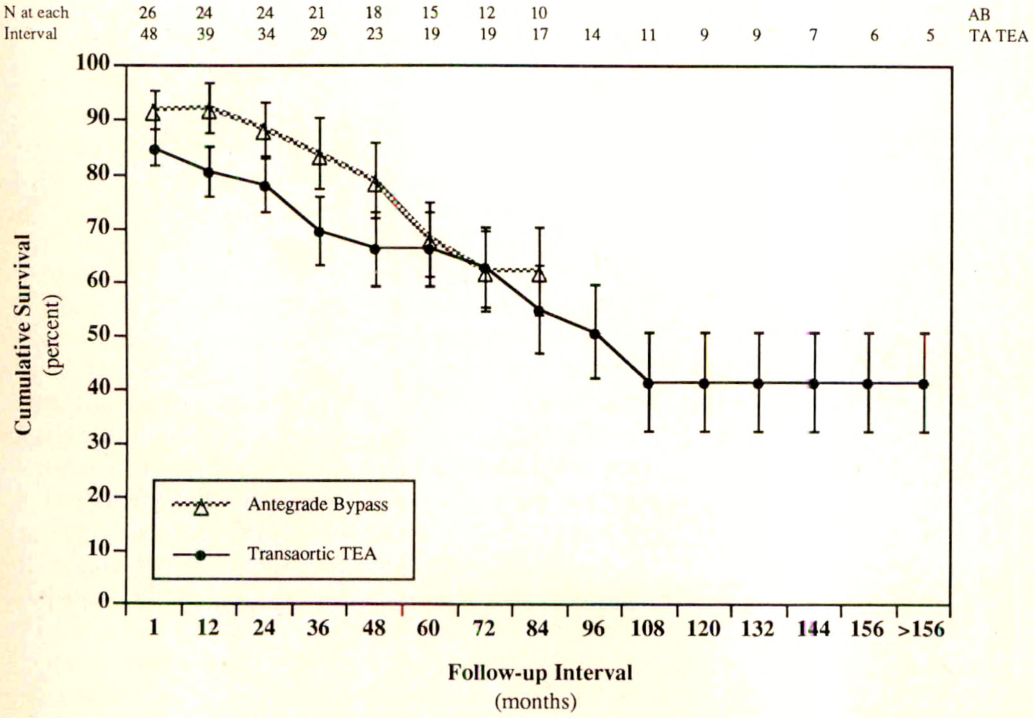
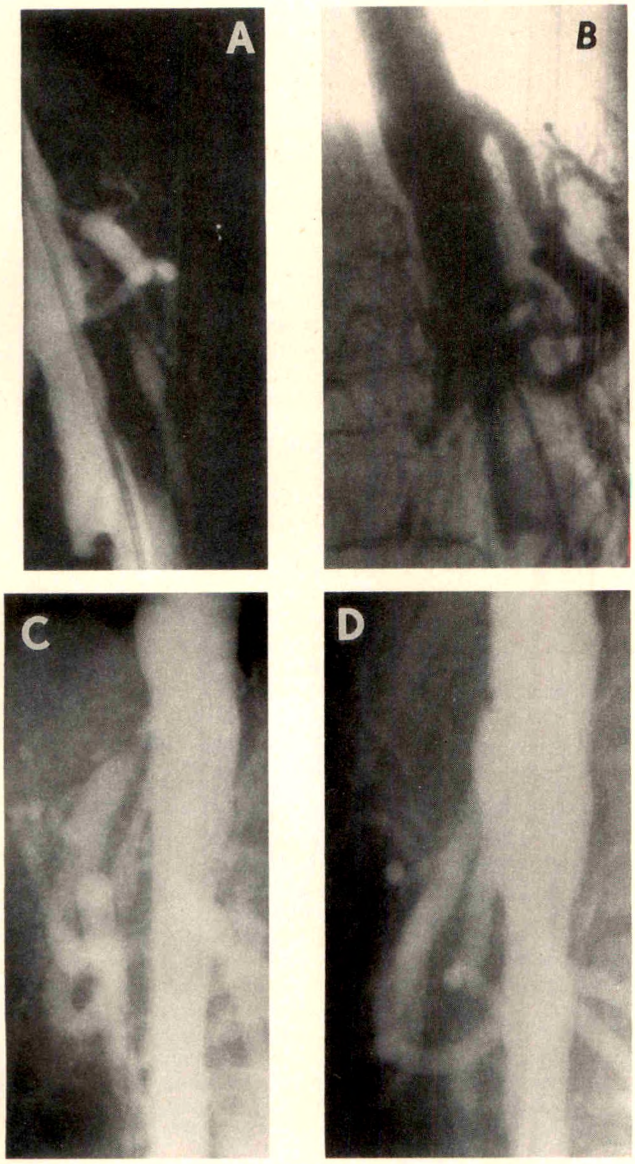


FIG. 11. Life table representation of the cumulative patient survival after antegrade bypass (AB) and transaortic endarterectomy (TA TEA). All SE less than 10%.



FIGS. 12A–D. Preoperative (A) and postoperative (B) arteriograms from a patient who underwent antegrade bypass to the celiac artery showing a widely patent bypass graft in an asymptomatic patient. Preoperative (C) and postoperative (D) arteriograms from a patient who underwent transaortic endarterectomy of both the celiac and SMA showing widely patent native vessels, also in an asymptomatic patient.

saortic endarterectomy does require special expertise and consequently few surgeons have adopted this approach to the treatment of visceral atherosclerosis. Those few other groups who have used this technique report results similar to ours.[34]

Antegrade Prosthetic Bypass

It seems obvious that the durability of the bypass is favored when the site of origin (distal thoracic aorta) is disease free at the time of implantation and at little risk for disease progression during the life of the patient. The antegrade orientation of these grafts allows for very short segment conduits, which have no propensity to kink and which may actually endothelialize over much of their length. This orientation, combined with an end-to-end distal anastomosis, may minimize flow abnormalities reported to contribute to intimal hyperplasia. Although the concept of antegrade visceral grafts was introduced in 1977[27] and has recently been endorsed by others,[35,36] most recent series of visceral revascularization continue to show a marked predominance of retrograde visceral bypass grafts.[33,36–40] Crude outcome data are available from most of these series, but life-table presentation of the duration of symptom relief and survival are not. Furthermore most of the series do not analyze the outcome data according to the type of revascularization used and some series combine patients with acute symptoms, chronic symptoms, and no symptoms. Thus the durability of retrograde bypass grafts for chronic visceral ischemia remains somewhat undefined.

The perioperative mortality rate for this study group is comparable to that reported in the literature. It is somewhat surprising that there was no significant difference in the perioperative mortality rate between the two treatment techniques, despite the fact that transaortic TEA required more extensive exposure, involved multiple visceral artery repairs more often than did antegrade bypass and was more often combined with other vascular repairs (n = 25, 52.1%) than antegrade bypass (n = 3, 11.5%). Because our two operative groups were comparable in terms of the overall patient profile, risk factors and associated illnesses, these results cannot be attributed to selection of better-risk candidates for transaortic TEA. One factor that may have contributed to reducing the transaortic TEA mortality is the increased use of the transabdominal approach. This certainly reduces the length and magnitude of the operation and ultimately may improve the overall complication rate among transaortic TEA patients.

Because coexistent coronary artery disease largely determines the morbidity and mortality rates of patients undergoing vascular reconstruction, we attribute much of this improved perioperative survival to the use of intraoperative transesophageal echocardiography. This method of cardiac monitoring allows accurate, rapid real-time detection of volume changes as well as the identification of ischemia-induced cardiac wall motion abnormalities. Prompt correction of volume changes and relief of myocardial strain, which often accompanies supraceliac aortic clamping, can prevent the consequences of myocardial ischemia in the postoperative period. Despite 25- to 30-minute transient supraceliac aortic occlusion in both treatment groups, there were only five perioperative cardiac events (6.8%), only one of which was fatal.

We also found no difference in the long-term relief of symptoms achieved with these two operative approaches. After 5 years of follow-up, 86% of patients in each operative treatment group remained symptom free. Of equal interest was the fact that six of the nine patients with recurrent symptoms were again rendered asymptomatic by repeat visceral revascularization (n = 5) or short-segment bowel resection (n = 1). Thus, during this extensive follow-up interval, only three patients (4.8%) experienced fatal recurrent visceral ischemia. We attribute these durable results to selection of the optimal revascularization technique and to intraoperative assessment of the reconstruction. The endarterectomy technique allows complete removal of all aortic and visceral atheroma, while the bypass technique provides antegrade flow from disease-free proximal aorta to disease-free distal visceral artery. The routine use of intraoperative duplex scanning to visualize the reconstructions and assess flow through the conduit or through the disobliterated native arteries ensures that the reconstruction will be technically perfect at the conclusion of the procedure. Undoubtedly this contributed significantly to the low incidence (n = 2) of early revascularization failures in our series because these are almost always due to a technical problem. Of interest many perioperative deaths reported by other groups resulted from early revascularization failure and intestinal infarction. We did not encounter that in this series. None of our perioperative deaths was due to failure of the visceral repair. Thus intraoperative Duplex scanning contributes importantly to perioperative survival and sets the stage for prolonged reconstruction patency.

The equivalent results seen in these two groups would suggest that durable relief of symptoms does not correlate with the number of visceral arteries repaired, in contrast to earlier published work.[41] Patients in the transaortic TEA group almost invariably had two or more visceral vessels endarterectomized, while nearly one half of the patients in the antegrade bypass group had only one diseased artery bypassed. Rather it would seem that the best way to insure long-term survival and durable relief of symptoms is to select the optimal operative technique, use aggressive intraoperative cardiac monitoring, and achieve technical perfection in the revascularization.

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Discussion

Dr. John E. Connolly (Irvine, California): Dr. Stoney and I have shared a common interest in reconstructive procedures for chronic intestinal ischemia for a number of years.

I looked up the paper that he gave in 1977 before this organization, and I do not know whether any of you read over the discussions you have given years before, but it can be very interesting. I would like to quote what I said about the trapdoor procedure at that time.

> I would think that the trapdoor method suggested by the authors would be applicable in some patients but not in most patients, and I would think in the average surgeon's hands it would be preferable to use bypass grafts into the distal mesenteric vessels and stay away from such a major procedure on a dangerous portion of the proximal abdominal aorta.

In talking to colleagues over the years, I believe that what I said then continues to be the common opinion of most vascular surgeons regarding the trapdoor procedure. I have a confession to make, however. After the meeting in 1977, I went home and thought about the trapdoor approach and decided to try it. I used an oblique abdominal incision with a short extension into the eighth intercostal space, the same approach that we had been using for years previously to perform direct retroperitoneal operations on the renal artery.

I was amazed how easy the trapdoor procedure was once you got the exposure. It was very much like a carotid endarterectomy, except that you did not have to worry about whether to shunt. The 15 or 20 minutes required to do the endarterectomy is about one third of the time that the kidney flow can be safely occluded, especially if you place ice slush in the retroperitoneal area next to the kidneys.

I have now performed seven trapdoor operations without any complications or known late patency failures and think it is the procedure of choice for revascularization for chronic intestinal ischemia in most patients. I only wish that more patients in our area with chronic intestinal ischemia were diagnosed before gut gangrene developed, as this is one of the most completely corrective procedures without the need of bypassing graft that I have had the satisfaction of doing in vascular surgery.

The only modification is that I find it is usually easier to include the left renal artery in the trapdoor incision whether or not one needs an endarterectomy of that artery. I have not tried the trapdoor operation completely transabdominally, and maybe this approach described today will encourage trial of the trapdoor operations by vascular surgeons who do not feel comfortable with the standard thoracoabdominal retroperitoneal approach. I think that they would find that the method of direct endarterectomy is more totally corrective of the disease that we are dealing with and will provide higher long-term patency.

Finally I would like to congratulate Dr. Cunningham on his presentation and Dr. Stoney on his pioneering contributions to this field.

Dr. Michael E. DeBakey (Houston, Texas): I would like to make a few comments about this interesting pattern of arterial occlusive disease. In our experience we found some aspects of this form of atherosclerosis to be different from the regular patterns of atherosclerotic occlusive disease. As you know atherosclerotic occlusive disease that produces clinical manifestations of ischemia occurs in four major arterial beds of the body, and the interesting point is that, in any patient population, this form of the disease is the least frequent—in our own experience about 3% of the total patient population.

There are other interesting aspects of this disease. For example as Dr. Cunningham pointed out, in this group of patients—and this was true in our patients as well, and I think in most other studies—the predominant sex is the female, whereas in almost every other form of atherosclerotic occlusive disease, the male sex is predominant.

Another interesting aspect is related to the risk factors. If for example we compare this pattern of disease with coronary atherosclerotic occlusive disease, we find that the incidence of the primary risk factors, such as tobacco smoking, hypertension, and hyperglycemia, are different. In this pattern of the disease, only 17% had hypercholesterolemia, and this observation has been made by us as well—less than 20% in our experience—whereas in coronary atherosclerotic disease, the presence of hypercholesterolemia is at least twice or three times that. It is closer to 60% to 70%.

Hypertension also is more common in the other patterns of the disease than it is in this group.

It also should be pointed out that clinical manifestations develop most often and almost invariably when more than one of the vessels are involved. Usually both the celiac and superior mesenteric arteries are involved in production of clinical manifestations.

As far as the technique is concerned, our experience has led us more to use of the bypass procedure than to endarterectomy. I believe, however, that the transabdominal approach to endarterectomy is preferable to the thoracoabdominal approach that we originally described.

The results, nevertheless, are equally good, but I think the reason that they are is that in the hands of such experienced vascular surgeons as Dr. Stoney and his associates gratifying results can be expected in 95% of the patients.

With regard to duration of survival, the results presented here are excellent. A recent analysis of our own experience with these patients, most of whom had the bypass procedure, showed that the Kaplan-Meier survival curve for 10 years was about 70%, and for 20 years was a little more than 50%. So we can expect good results when the procedure is performed as excellently as has been presented by Dr. Stoney and his associates.

I also want to thank Dr. Stoney for letting me review his manuscript. It is an excellent study and most useful in presenting the kind of treatment that is indicated for this form of disease, despite the fact that is it relatively uncommon.

Dr. Victor M. Bernhard (Tucson, Arizona): This certainly is one of the largest, if not the largest, reported experiences relating to the surgical management of ischemia of the intestine, and the results presented strongly indicate that revascularization is a durable method of management.

My questions to the authors relate to the basis for their choice of operative technique. Most of us grew up with the concept of performing a bypass using the retrograde approach, especially when dealing with combined infrarenal aortic disease, either aneurysm or atheromatous obstruction. This is the most common setting in my practice and I have usually employed retrograde bypass with prosthetic conduits rather than veins under these circumstances. Conversely I like and have used the prograde approach either with bypass or with endarterectomy when the infrarenal aorta does not require repair and have found that both of these techniques are extremely satisfactory.

You suggest that the prograde approaches are better; nevertheless the data in the literature are anecdotal. Because you have experience with other techniques, what data do you have that support your preferences for the prograde rather than the retrograde approach? What do you do for those patients who require combined infrarenal aortic reconstruction and visceral revascularization? Do you avoid taking a bypass off the infrarenal aortic reconstruction and use a separate approach at the diaphragmatic crural area of the aorta?

With regard to bypass *versus* endarterectomy, do you have criteria that direct you to perform one rather than the other? Is this decision based on a preoperative assessment of collaterals and the ability to identify aortic wall disease by computed tomography (CT) or magnetic resonance imaging (MRI)? We have found that these studies may be helpful.

Finally I noticed that you obtained a more complete revascularization with endarterectomy, as both the celiac and the superior mesenteric vessels are repaired, whereas with the bypass technique, about half of your patients received only single-vessel repair. Was it merely the technical clumsiness of the problem, or were there certain criteria that made you decide that you could get away with single-vessel, usually celiac, repair without bothering to also include the superior mesenteric?

DR. GARLAND D. PERDUE, JR. (Atlanta, Georgia): I will try to show the wisdom and perspicacity to agree with most of the points. I will comment on our own experience, which is confirmatory, but with some slight differences.

My colleagues reported a series of 41 patients, I believe it was, with a mortality rate of two of those patients, about 5%, over a 5-year period some 2 or 3 years ago, and did report a rather eclectic choice of technical procedures. I would acknowledge that we still use some retrograde bypasses. My personal preference, when feasible, is for endarterectomy or for antegrade bypass, but my colleagues, when they found infrarenal aortic disease requiring prosthetic replacement, elected retrograde bypass as well.

I think it is correct that our anesthesiologists have taken a certain adventure out of the operative management through their methods for afterload reduction and intraoperative monitoring. We would agree that completion imaging is very important to assure success of the operation.

I would like to raise some questions about preoperative management. We went through an era of intensive preoperative nutritional restoration, had a few patients who developed acute ischemic symptoms during this wait period, and have largely abandoned that, choosing instead to let the patients eat themselves back to health, I guess, after revascularization.

I would like to inquire of this group if they still go through any special preoperative nutritional preparation or allow it to occur postoperatively?

I would like, perhaps in some anticipation of a succeeding paper on the program, to inquire if any attention is paid to preoperative optimization of ventricular function before undertaking the procedure?

DR. NORMAN R. HERTZER (Cleveland, Ohio): Together with Jack Wylie, Dr. Stoney and his group have pioneered mesenteric vascular reconstruction in the United States of America and their results are exceptional.

Secondly I would like to describe what must be an unusual complication of trapdoor transaortic endarterectomy in my own experience. About 2 days after such an operation, we were forced to re-explore a patient for bleeding and found that the spleen was easily three times normal size and contained a stellate capsular rupture. This did not appear to be an iatrogenic injury, and I presumed that the engorgement of the spleen in this case could be correlated with the hyperemic, edematous foot after limb salvage revascularization.

Has the UCSF group ever encountered this complication, or was this maturing experience mine alone?

DR. LARRY HOLLIER (New Orleans, Louisiana): However I cannot help but feel that the benefits that they have ascribed to the techniques are probably misplaced. I do not think that it is the techniques alone that have really allowed these excellent results to occur; it has been the excellent surgical judgment of Dr. Stoney and his colleagues in selecting which patients get this specific procedure.

The authors have left out 11 patients who had visceral revascularization using techniques that they can no longer recommend. Unfortunately we all see patients in whom the condition of the aorta or the extent of the visceral occlusive process is such that neither one of these techniques that they advocate are really suitable. I would be interested in hearing the types of procedures that were excluded and the results of those procedures.

DR. CHRISTOPHER CUNNINGHAM (Closing discussion): We agree with Dr. DeBakey that visceral atherosclerosis is unusual in its infrequency, female gender predominance and—at least in our group—its low incidence of associated diabetes mellitus. However, we found both smoking and hypertension to be very prevalent among our patients. Although a formal diagnosis of hypercholesterolemia was very rare among our patients, we have been impressed by the fact that most of these patients have serum cholesterol levels at the upper limit of the so-called normal range. This is in spite of the fact that these patients are all essentially starved. Thus it is our belief that these patients are actually relatively hypercholesterolemic.

Dr. Bernhard, we have not performed enough retrograde bypasses ourselves to directly compare the results obtained with that technique to our two preferred techniques. We believe that the results that we are able to obtain with antegrade bypasses would be hard to improve on. However we agree with you that a direct comparison of antegrade and retrograde visceral bypasses would be interesting. For those patients who need infrarenal aortic repair, we would most often perform transaortic TEA because we think it actually facilitates the infrarenal aortic reconstruction. Consequently we do not need to originate any visceral bypass from the infrarenal graft. In those infrequent instances where a visceral endarterectomy was planned and/or attempted but could not be completed and the infrarenal aorta required reconstruction, we would probably still use an antegrade bypass from the supraceliac aorta, not from the infrarenal graft. This does not require a separate transcrural approach to the aorta at the diaphragm, because we already would have exposed that area in preparation for the initially planned TEA, and the exposure would have been extended to include the infrarenal repair. We do not have absolute criteria for choosing between bypass and endarterectomy. Factors that encourage the use of endarterectomy include the need for associated renal and/or aortic reconstruction and visceral disease limited to the vessel orifices. On the other hand, antegrade bypass is favored when there is transmural calcification and when the visceral lesions are very extensive. Aside from these specific factors, surgeon preference may exert the definitive influence. Imaging studies and presence of collateral vessels have not played a role in the choice of reconstruction technique. We have not been able to identify discretely what makes it safe to limit revascularization to a single vessel. However the status of collateral flow to the vessel that is not repaired is important in that decision.

Dr. Perdue, we no longer routinely attempt nutritional repletion in these patients before operation. However we still do this in selected patients. We have intermittently tried to optimize cardiac performance preoperatively in all of our patients undergoing aortic reconstruction, not just those patients facing visceral repair. At times this included preoperative admission to a unit bed and placement of arterial and Swan-Ganz lines for determination of cardiac output, followed by volume loading and/or afterload reduction to improve cardiac function. We do not have the facilities to do this routinely and thus we rarely resort to this type of preoperative preparation at this time. However almost all of these patients undergo preoperative echocardiography and persantine-thallium testing. Subsequent to that it is not uncommon for cardiac medications to be started or adjusted.

Dr. Hertzer, as I was presenting this paper, we were in the midst of treating our first complication of this type, unfortunately involving the liver. We agree with you that this seems to be a reperfusion edema type of injury, but we cannot explain its mechanism—or for that matter why we have not seen it more often. Unfortunately the development of such a problem in the liver is substantially more life-threatening.

Dr. Hollier, the techniques used in the excluded patients included transarterial endarterectomy, reimplantation, patch angioplasty, transposition, and one lonely retrograde bypass. In these patients the reconstruction usually used some combination of these approaches. In this group of 11 patients—9 of whom were operated upon before 1970—there was one perioperative death (9.1%). During an average follow-up interval of 19.9 months, recurrent symptoms of visceral ischemia developed in two patients (18.2%). Symptoms resolved spontaneously in one patient, and she remains asymptomatic 21 years later. One patient underwent redo celiac revascularization 3 years postoperatively for recurrent symptoms. His symptoms improved greatly, but did not resolve completely until his duodenal ulcer was diagnosed and treated medically. Subsequently he remained asymptomatic until his death, which was due to a myocardial infarction, 15 years later. Seven perioperative or follow-up angiograms showed that all of the operated arteries were patent; three studies showed one (N = 2) or two (N = 1) operated arteries in which stenoses or occlusion had developed.

The authors thank the Association for the opportunity to present this work and all of the discussants for their kind comments.

Preoperative Optimization of Cardiovascular Hemodynamics Improves Outcome in Peripheral Vascular Surgery

A Prospective, Randomized Clinical Trial

JON F. BERLAUK, M.D.,* JEROME H. ABRAMS, M.D.,† IAN J. GILMOUR, M.D.,* S. RHIANNON O'CONNOR, M.D.,*
DAVID R. KNIGHTON, M.D.,† and FRANK B. CERRA, M.D.†

From the Departments of Anesthesiology and Surgery,†*
University of Minnesota, Minneapolis, Minnesota

The hypothesis that optimizing hemodynamics using pulmonary artery (PA) catheter (preoperative 'tune-up') would improve outcome in patients undergoing limb-salvage arterial surgery was tested. Eighty-nine patients were randomized to preoperative tune-up either in the surgical intensive care unit (SICU) (group 1) or the preinduction room (group 2) or to control (group 3). The tune-up consisted of fluid loading, afterload reduction, and/or inotropic support to achieve predetermined endpoints. Patients with a PA catheter had significantly fewer adverse intraoperative events ($p < 0.05$), less postoperative cardiac morbidity ($p < 0.05$), and less early graft thrombosis ($p < 0.05$) than the control group. The overall study mortality rate was 3.4%, with a mortality rate of 9.5% in the control group and 1.5% in the PA catheter groups. There were no differences in ICU length of stay (LOS), hospital LOS, or total hospital costs, although the percentage of cost from complications was higher in group 3 ($p > 0.05$). In this group of patients, preoperative cardiac assessment and optimization is associated with improved outcome.

PULMONARY ARTERY (PA) CATHETERIZATION for the preoperative evaluation of elective surgical patients remains controversial.[1] Nevertheless, in certain groups of surgical patients, the PA catheter has revealed abnormal cardiovascular hemodynamics, which are not reliably predicted by clinical examination.[2–4] Patients undergoing surgery for peripheral vascular disease (PVD) tend to be elderly, diabetic, and are known to be at high risk for cardiovascular complications.[3,5–8] Although routine PA catheterization of this group of patients has been recommended,[9] preoperative hemodynamic assessment is not standard practice despite a 40% incidence of abnormal left ventricular function (LVF).[3]

While the risks of PA catheterization are well known,[10–19] reports of benefits from pulmonary artery pressure monitoring, especially in critically ill patients, are also accumulating.[20–28] However no controlled, prospective, randomized clinical trial has been performed to show that PA catheterization changes outcome in patients in whom it is used.[29–31]

We designed our study to answer two questions:

1. In patients with PVD, does the use of a PA catheter to 'optimize' LVF and oxygen transport (tune-up) preoperatively influence the clinical outcome?
2. How important is the length of time allowed for the preoperative tune-up?

Materials and Methods

Study Design

A randomized, prospective, clinical trial was approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota. Patients from a single surgical service, scheduled to receive an *in situ* vein graft bypass for limb salvage, made necessary by peripheral lower-extremity atherosclerotic arterial occlusive disease, were eligible to participate. All operations were performed by a single surgeon (DR Knighton). Patient eligibility was determined by the surgical intensive care unit (SICU) anesthesiologist (JF Berlauk/IJ Gilmour). The randomization for all study groups was generated by a random-number generator (Statworks®). Eligible patients were entered consecutively in order of their appearance on the surgical schedule. Patients with the following problems were excluded because these clinical settings have literature documentation that supports the use of the PA catheter during necessary surgical intervention:

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Address reprint requests to Frank B. Cerra, M.D., Department of Surgery, University of Minnesota, Box 42 UMHC, 406 Harvard St. SE, Minneapolis, MN 55455.

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1. Myocardial infarction (MI) within the previous 3 months.[32–36]
2. Coronary artery bypass graft (CABG) within the previous six weeks.[37]
3. Uncompensated congestive heart failure (CHF),[7,38–41] identified clinically by pedal edema, jugular venous distention, pulmonary rales, and chest x-ray (CXR) findings of pulmonary edema.
4. Severe valvular heart disease,[38,42] defined as symptomatic aortic or mitral valvular disease.
5. 'Unstable' angina, defined as angina at rest or a recent change in an angina pattern.

After written consent was obtained, eligible patients were randomized into two groups. Group 1 received a PA catheter at least 12 hours before operation. If cardiovascular physiologic abnormalities were discovered by PA catheter measurements, these patients underwent pharmacologic manipulation to 'optimize' intravascular volume and cardiac function, as described in 'Preoperative Treatment Regimens.' The remaining patients were randomized further into two groups. Group 2 patients received a tune-up within 3 hours of surgery. This group was allowed less time for hemodynamic assessment, but therapeutic goals and endpoints were the same as for group 1. The control patients (group 3) did not receive a PA catheter before operation nor did they undergo a tune-up.

All patients received a radial artery catheter for continuous blood pressure (BP) monitoring if the Allen's test demonstrated good collateral flow. An automated sphygomanometer (DINAMAP®, Criticon, Tampa, FL) was used on patients with compromised flow.

All patients were given general anesthesia using a standardized technique. A single SICU anesthesiologist directed the intraoperative care. After recovery from anesthesia, all patients were transferred to the SICU. Surgical intensive care unit care was provided to all patients in the study by the same team.

Preoperative Treatment Regimens

Group 1. Patients were transferred to the SICU approximately 12 hours before surgery. All cardiovascular medications were discontinued except for clonidine, beta-blocking agents, and digoxin. Pulmonary artery and arterial catheters were placed by or under the supervision of SICU attending staff or senior surgical fellows. Most of the PA catheters were inserted *via* the right internal jugular vein. Mean BP, heart rate (HR), central venous pressure (CVP), pulmonary artery wedge pressure (PAWP), and cardiac output (CO) were measured. All measurements in the SICU were done by trained monitoring technicians. From these measurements derived variables were calculated: stroke volume, systemic vascular resistance (SVR), pulmonary vascular resistance, and cardiac index (CI). We attempted to follow an algorithm for hemodynamic intervention (Fig. 1). Hemodynamic studies were repeated after each intervention and decision making was coordinated with the SICU staff. The endpoints of successful hemodynamic intervention were defined as:

1. $8 \leq \text{PAWP} < 15$ mmHg.[43,44]
2. $\text{CI} \geq 2.8$ L/min/m^2.[45]
3. $\text{SVR} \leq 1100$ dyne-sec cm^{-5}.[43]

These end-points were chosen because of their documented correlation with outcome. When these goals were achieved, no further intervention was attempted and intravenous crystalloid was infused at 1 to 2 mL/kg/hr. If these endpoints could not be achieved, the surgeon and SICU staff decided whether the patient would tolerate the anticipated surgical procedure.

Group 2. These patients had invasive catheters placed by an SICU anesthesiologist less than 3 hours before surgery in a preinduction room (PIR). Although less time was available, the protocol and endpoints were otherwise identical to those used for group 1.

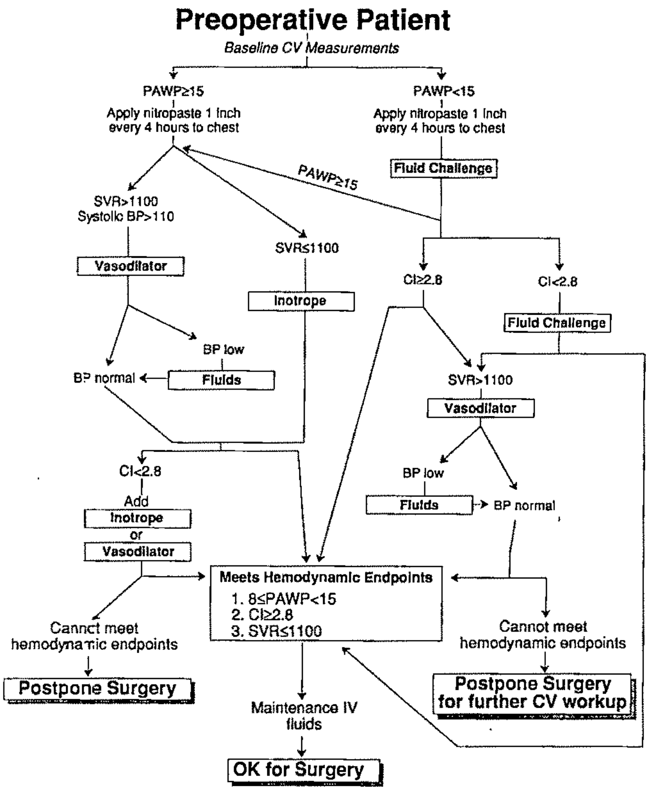

FIG. 1. Algorithm for preoperative cardiovascular tune-up. CV measurements were repeated after each intervention. Inotropes: dobutamine or dopamine. Vasodilators: nitroglycerin or nitroprusside. CV, cardiovascular; PAWP, pulmonary artery wedge pressure; SVR, systemic vascular resistance; CI, cardiac index. Measurement units are mmHg for pressure, dyne-sec cm^{-5} for resistance and L/min/M^2 for CI.

Group 3. An arterial catheter was placed before anesthesia. Most of these patients received a CVP catheter as well. A PA catheter was not used during operation unless a complication occurred that required its use. Under these circumstances the PA catheter was inserted by the attending anesthesiologist during operation or by the SICU staff after operation.

Intraoperative Management

A single anesthetic protocol was followed for all study groups. In the PIR all monitors were applied and necessary vascular access was established. Baseline BP (mean) and HR were measured while the patient was at rest. Premedication was midazolam (Versed®, Roche) (0.5 to 4.0 mg administered intravenously) or fentanyl (1.5 to 2.0 μg/kg administered intravenously) as required.

Anesthesia was induced with midazolam (Versed®) (0.15 to 0.30 mg/kg administered intravenously) and intubation was facilitated by vecuronium (Norcuron®, Organon) (0.1 mg/kg administered intravenously). Lidocaine (1 to 1.5 mg/kg administered intravenously) bolus was given 90 seconds before intubation. Anesthesia was maintained with isoflurane (Forane®, Anaquest, Madison, WI), air, and oxygen. Additional fentanyl and vecuronium were given as required.

Intraoperative monitoring included the V_5 lead of the electrocardiogram (EKG), pulse oximetry, and end-tidal CO_2. Adverse intraoperative events were defined as:

1. Decrease in mean BP ≥ 30%[32,46] below baseline.
2. Increase in the HR > 30%[41,46] above baseline.
3. Arrhythmia,[32,47] defined as any new rhythm disturbance.
4. ST changes,[48] defined as ST depression > 1 mm or T-wave inversion.

These adverse intraoperative events were chosen because there is documentation in the literature that they correlate with increased perioperative morbidity. Intraoperative events were recorded by the anesthetist on the anesthetic record and tabulated later. Those patients with a PA catheter had a hemodynamic profile measured at least every 2 hours during operation. The hemodynamic endpoints previously defined were maintained during anesthesia in groups 1 and 2.

Postoperative Management

All patients were continuously monitored in the SICU for at least 18 hours after operation. Major clinical events recorded for the study included:

1. Pulmonary edema by CXR or clinical examination.
2. Acute MI defined by new Q waves more than 0.03 seconds or creatinine phosphokinase MB isoenzyme (CPK_{MB}) more than 5%.
3. Postoperative arrhythmias requiring anti-arrhythmic agents and/or prolonging the SICU stay more than 36 hours.
4. Acute renal failure (ARF), defined as urine output less than 0.5 mL/kg/hr for 5 hours and/or a change in baseline serum creatinine more than 0.5 mg/%.
5. New wound infection.
6. Sepsis.
7. *In situ* graft thrombosis.

Criteria for discharge from the SICU were:

1. Hemodynamics stable (mean BP more than 90 mmHg, HR less than 100) for 18 hours.
2. Extubated, PaO_2 more than 65 torr on $FiO_2 \leq 0.3$.
3. Temperature less than 100.5 F measured orally.
4. Urine output ≥ 0.5 mL/kg/hr with a stable creatinine.
5. CPK_{MB} less than 5% of total CPK.
6. EKG unchanged from admission or unchanged for 3 days.
7. Stable EKG rhythm.

Laboratory Studies

Baseline studies for all patients included an blood chemistry admission battery, EKG, CXR, 12-hour creatinine clearance, and serum CPK_{MB}. A CPK_{MB} was drawn at the end of the preoperative assessment of group 1. In all groups a CPK_{MB} was drawn at the end of surgery and every 8 hours after operation. After operation an EKG, CXR, and 12-hour creatinine clearance were performed on all patients.

Data Management

Data were collected prospectively and entered into a computerized database. Demographic and chemistry data were analyzed by analysis of variance. The clinical outcome variables were analyzed by the chi square (X^2) technique for nonparametric data. The primary clinical outcome variable was a cardiovascular complication such as congestive heart failure, arrhythmia, or myocardial infarction. Secondary outcome variables were immediate postoperative graft thrombosis and adverse intraoperative events. Significance was defined as $p < 0.05$. Data were analyzed periodically for significant differences in the primary clinical outcome variable. When the between-group differences reached $p < 0.05$ for this variable, the study was terminated.

Results

Eighty-nine patients were enrolled into the study from October 1986 to January 1990. Enrollment continued until the clinical outcome differences between groups were statistically significant. By history 11% of the study patients had angina, 27% had a previous MI, 15% had CHF, 50% had hypertension, 72% had diabetes mellitus, and

TABLE 1. *Patient Characteristics*

	Treatment Groups		
	PA Catheter		No PA Catheter
	1	2	3
Age (yrs)	67.8 ± 12.4*	61.5 ± 13.5	65.8 ± 11.9
Male/female	22/23	16/7	13/8
No.	45	23	21
Prior history of			
Angina	8†	2	0
MI	14	5	5
CHF	10	1	3
HTN	26	9	10
DM	34	16	14
CVA	12	4	5

* Mean ± SD.

† $p < 0.05$ compared to group 3.

MI, myocardial infarction; CHF, congestive heart failure; HTN, hypertension; DM, diabetes mellitus; CVA, cerebrovascular accident.

23% had a previous cerebrovascular accident. There were 45 patients in group 1, 23 patients in group 2, and 21 patients in group 3 (Table 1). There were no statistically significant differences between groups except that 80% of the patients with a history of angina were randomized to group 1 ($p < 0.05$). When groups 1 and 2 combined (patients with PA catheters) were compared to group 3 (control patients), there was no statistical difference between the groups except for angina ($p < 0.05$). No patient randomized to the control group had a history of angina (Fig. 2).

Preoperative Hemodynamic Assessment

While patients with a history of CHF appear to predominate in group 1 compared to group 2 (22.2% *versus* 4.3%), Table 2 shows that more patients in group 2 had an elevated baseline pulmonary capillary wedge pressure (26.1% *versus* 17.8%) and demonstrated left ventricular dysfunction (26.1% *versus* 15.6%) in response to a fluid challenge.

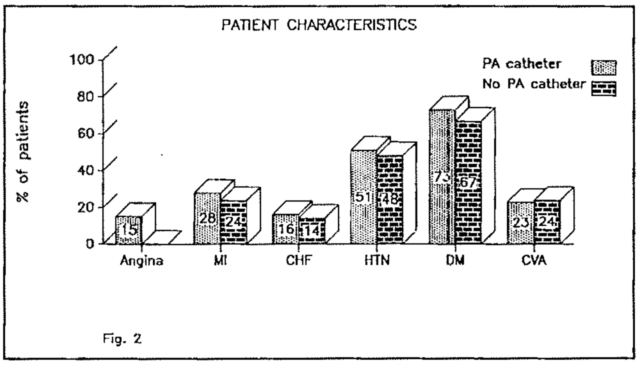

FIG. 2. Patient characteristics.

TABLE 2. *Results of Hemodynamic Assessment and Intervention*

	Treatment Group	
	1	2
Initial Assessment		
No. of patients	45	23
SVR > 1100 dynes-sec cm^{-5}	30	11
CI < 2.8 L/min/M^2	7	2
PAWP ≥ 15 mmHg	8	6
PAWP ≥ 15 mmHg after IV Hydroxyethylstarch	7	6
Intervention		
No. of patients achieving all hemodynamic endpoints		
A	14	11
B	14	4
C	14	6
D	2	2
E	1	0

A: Baseline hemodynamics meet endpoints.
B: IV fluids alone (colloid plus crystalloid).
C: IV fluids plus an inotrope OR vasodilator.
D: Inotrope AND vasodilator required.
E: Cannot achieve endpoints despite therapy.

In 25 of 68 patients (36.8%) who received a PA catheter, baseline cardiovascular (CV) measurements met our endpoints so nothing further was done (intervention A). Of these 25 patients, 1 (group 1) subsequently developed ARF, 1 (group 1) developed CHF, and 1 (group 2) had a postoperative graft thrombosis (Table 4). Eighteen of sixty-eight patients (26.4%) required fluids alone (intervention B) to achieve the endpoints. The remaining 25 of 68 patients (35.8%) with a PA catheter required inotropic and/or vasodilator agents along with intravenous fluid to achieve 'optimal' hemodynamic status (interventions C, D, and E). Only one patient from group 1 did not achieve the endpoints after all of the above interventions. In this patient the planned surgery was canceled and a less complicated procedure was performed under local anesthesia without complications.

We found no correlation between either the baseline hemodynamic profile or the hemodynamic response to therapy and subsequent complications in groups 1 and 2.

Intraoperative Events

Sixty-six of eighty-nine patients (74%) received anesthetic care from a single SICU anesthesiologist; 81 of 89 patients (91%) received anesthetic care from either of two SICU anesthesiologists.

After entry into the study, no patient was excluded but surgery was canceled for two patients in group 1, leaving 43 patients who completed the study (Table 3). One of these patients had a preoperative MI (see Complications section) and the other could not achieve the hemodynamic

TABLE 3. *Intraoperative Events*

	Treatment Groups		
	PA Catheter		No PA Catheter
Adverse Event	1	2	3
	n = 43	n = 23	n = 21
Incidence			
Tachycardia*†	5	4	4
Hypotension*†	16	6	10
Arrhythmia*†	9	5	8
	n = 8	n = 3	n = 9
Incidence in those patients with complication			
Tachycardia*†	1	1	1
Hypotension*†	2	1	4
Arrhythmia*†	1	0	3

* $p < 0.05$ for group 1 *vs.* 3.
† $p < 0.05$ for groups 1 and 2 *vs.* 3.

endpoints (see Preoperative Hemodynamic Assessment section). In addition one patient from each group had the vein graft bypass aborted during operation for technical reasons because of poor vessels. However their data are included in Table 3.

The control group (group 3) experienced a significantly higher incidence of intraoperative events (tachycardia, hypotension, and arrhythmias) as compared to either group 1 ($p < 0.05$), group 2 ($p < 0.05$), or combined groups 1 and 2 ($p < 0.05$). In addition these adverse intraoperative events correlated with major perioperative complications in the control patients ($p < 0.05$) (Table 3). Overall patients who received a PA catheter before operation were more stable during operation. All adverse events were less than 10 minutes in duration. Because permanent real-time recordings of intraoperative events were not obtained, observations of ST-T changes were considered unreliable and eliminated from analysis.

Complications

The overall complication rates were 17.8% (8 of 45 patients) in group 1, 13% (3 of 23 patients) in group 2, and 42.9% (9 of 21 patients) in group 3 (Table 4). The mortality rate was 2.2% (1 of 45 patients) in group 1, 0% in group 2, and 9.5% (2 of 21) in group 3. The morbidity rate associated with the preoperative tune-up, including placement of the PA catheter, was 4.4% (3 of 68). Morbid events included two preoperative MIs (see below) and one pneumothorax.

Group 1. Eight hemodynamic complications occurred in group 1 (Table 4). Two patients in group 1 had non–Q-wave MIs during the tune-up. In one patient clinical symptoms, hypotension, and EKG changes called attention to the problem and surgery was canceled. A CPK_{MB} fraction of 22% of total CPK later confirmed the clinical

TABLE 4. *Hemodynamic Profile of Patients with Complications*

				Initial Assessment				
	Patient	Sex	Complication	CI < 2.8	PAWP ≥ 15	SVR > 1100	After Fluid PAWP ≥ 15	Intervention Group
Group 1								
	1	M	ARF	(−)	(−)	(−)	(−)	A
	2	F	CHF	(−)	(−)	(−)	(−)	A
	3	F	Death	(−)	(−)	(+)	(+)	C
	4	M	Graft thrombosis	(−)	(−)	(+)	(−)	C
	5	F	Postop MI	(−)	(−)	(+)	(+)	C
	6	M	CHF	(−)	(+)	(−)	Not done	C
	7	M	Preop MI	(+)	(+)	(+)	Not done	D
	8	F	Preop MI	(−)	(+)	(+)	Not done	C
Group 2								
	1	M	CHF	(−)	(−)	(+)	(−)	B
	2	M	CHF	(−)	(+)	(+)	Not done	D
	3	M	Graft thrombosis	(−)	(−)	(−)	(+)	A
Group 3								
	1	F	Arrhythmia					
	2	F	Graft thrombosis					
	3	F	Graft thrombosis					
	4	M	Graft thrombosis					
	5	M	Death (cardiac arrest)					
	6	F	Death (cardiac arrest)					
	7	M	Postop MI					
	8	F	ARF, CHR					
	9	M	Graft thrombosis					

See Table 1 for abbreviations.

diagnosis of subendocardial MI. A second patient had no signs or symptoms of MI and underwent surgery with no complications. Only later was the MI appreciated when the preoperative CPK_{MB} returned at 15% of total CPK. The data was analyzed both with and without these two patients. The results were unaffected by their inclusion or exclusion from the data analysis.

Six other patients had other perioperative CV complications. One patient developed ARF after operation. This was attributed to a large intravenous contrast medium load. The renal failure resolved before the patient was discharged from the hospital. A second patient died on postoperative day (POD) 7 during emergency surgery to repair a ruptured common iliac artery. Percutaneous angioplasty had been performed on the vessel earlier that day. One patient suffered vein graft thrombosis about 36 hours after operation. Thrombectomy failed to restore blood flow and a below-knee amputation was performed on POD 3. Another patient had a non–Q-wave MI on POD 1 with CPK_{MB} enzyme elevation to 12.4% of total CPK. His recovery was not affected by complications. The last two patients developed mild CHF after operation; they were treated with fluid restriction, diuresis, and supplemental O_2. No evidence of MI was found in either patient.

Group 2. Three patients had postoperative hemodynamic complications in group 2 (Table 4). Two patients had CHF without evidence of MI. Both responded rapidly to fluid restriction, diuresis, and supplemental O_2. One patient developed an early postoperative graft thrombosis and underwent a successful thrombectomy about 2 hours after the initial operation.

Group 3. There were two postoperative deaths from cardiac arrest in group 3. One patient died on POD 6 after several days of refractory ventricular arrhythmias. Myocardial infarction could not be confirmed as the cause of her arrhythmias. No PA catheter was used in this patient. The second patient suffered cardiac arrest on POD 5 and could not be resuscitated.

There were eight other postoperative hemodynamic complications in seven patients in group 3 (Table 4). One patient had an acute MI on POD 2 and underwent an emergency five-vessel CABG on POD 3. A second patient had serious atrial and ventricular arrhythmias for 2 days after operation. These arrhythmias were successfully treated pharmacologically. No PA catheter was used in this patient. A third patient developed ARF and CHF during operation necessitating PA catheter insertion during operation. After operation the patient responded to dialysis and inotropic support. The ARF resolved; no MI was found. Four patients had graft thromboses within 36 hours of operation. Re-exploration of the surgical anastomosis and repeat angiograms revealed no technical problems. All patients had successful thrombectomy with restoration of blood flow and were placed on heparin. The grafts remained patent. None of these patients received a PA catheter.

Complications are summarized in Table 5. Both groups of patients with a PA catheter had significantly fewer adverse intraoperative events ($p < 0.05$), less postoperative cardiac morbidity ($p < 0.05$), and less early graft thrombosis ($p < 0.05$) compared to the control group. The mortality rate was 9.5% in the control group and 1.5% in the combined groups of patients with a PA catheter.

Other Analysis

There were no significant differences between groups in baseline blood urea nitrogen (BUN), serum creatinine (Cr), blood lactate, or serum potassium (K^+). There were no differences between groups in length of hospital stay (LOS) before surgery, LOS in the ICU, total hospital LOS, or total hospital charges (Table 6). Furthermore no differences were found between patients with a complication *versus* no complication, except control patients with complications spent 3.6 ± 2.9 days in the ICU compared to 1.9 ± 0.6 days if no complication occurred ($p < 0.05$). In addition the increased total hospital charges ($29,928 ± 14,078) for a complication in the control group offset any costs associated with PA catheter insertion in the SICU ($29,102 ± 13,207) or in the PIR ($23,770 ± 12,418) (Table 6)

Discussion

Does 'optimizing' cardiovascular hemodynamics using a PA catheter improve outcome? To test this hypothesis, we chose to study surgical patients with PVD, a group known to be at high risk for perioperative cardiovascular complications.[8] Because aortic and intraperitoneal surgery are associated with increased postoperative cardiac complications,[32,33,38,39] only patients with peripheral vascular surgery for limb salvage were included. To help decrease the number of confounding clinical variables, the study used a single vascular surgeon, a single anesthetic technique, and a single SICU service.

Using the PA catheter to optimize a patient's preop-

TABLE 5. *Complication Summary*

Characteristic	Group 1 (n = 45)	Group 2 (n = 23)	Group 3 (n = 21)
Intraoperative event (arrhythmia, tachycardia or hypotension)	30*	15*	22
Postop cardiac morbidity	3*	2*	5
Early graft thrombosis (<36 hr)	1*	1*	4
Death†	1 (2.2%)	0	2 (9.5%)

* $p < 0.05$ compared to group 3.

† 1.5% for groups (1 and 2 *versus* group 3 ($p = 0.08$).

TABLE 6. *Hospital Stay and Charges: Complication Versus No Complication (mean ± SD)*

	Treatment Groups		
	1	2	3
Hospital stay before surgery (day)	6.2 ± 4.9	5.9 ± 4.9	4.8 ± 3.8
ICU stay (day)	3.5 ± 2.0	2.5 ± 1.3	2.6 ± 2.1
No complications	3.1 ± 0.6	2.3 ± 1.2	1.9 ± 0.6
Complications	5.3 ± 4.3	3.7 ± 1.3	3.6 ± 2.9*
Hospital stay (day)	19.4 ± 11.6	18.0 ± 12.0	15.4 ± 7.5
No complications	19.1 ± 11.9	13.0 ± 5.1	13.4 ± 7.8
Complications	21.1 ± 9.9	18.8 ± 12.5	18.1 ± 6.4
Total hospital charge ($)	29,102 ± 13,207	23,770 ± 12,418	23,386 ± 12,303
No complications	26,916 ± 11,920	23,708 ± 12,880	18,479 ± 7750
Complications	39,211 ± 14,132	24,177 ± 8720	29,928 ± 14,078

* $p < 0.05$ compared to no complication in same group.

erative cardiovascular status is not new.[2] The catheter has been used in the preoperative assessment of patients undergoing hip pinning,[49] aortic aneurysm repair,[50,51] peripheral vascular surgery,[3] and other 'major' surgical procedures.[2,45,52–54] Therapeutic endpoints of the cardiovascular tune-up vary with the different studies. The endpoints for our therapeutic interventions were directed at (1) preoperative hypovolemia[2,43,53,55] and (2) improving myocardial performance to meet the increased respiratory, circulatory, and metabolic demands imposed on the postoperative patient.[43,56,57] Distal vascular surgery is not a high-risk procedure[38] so we chose normal values as therapeutic endpoints.

The demographics of our study group corroborate their high-risk status. Seventy-two per cent of the patients had DM,[33,39,40,58] 50% had hypertension,[32,33,58,59] 27% had a previous MI,[6,7,39,47,60] 15% had compensated CHF,[7,38–41] and 11% had angina.[6,33,39,47,58] These risk factors were evenly distributed between groups except that no patient with angina was randomized to the control group. Although the randomization sequence was performed at the beginning of the study, actual patient entry into the study was finalized the evening before surgery. Bias of the chief surgical resident, who determined the operating room schedule, may account for the lack of patients with angina in the control group. While stable angina is controversial as a predictor of cardiac morbidity in the noncardiac surgical patient,[61] its presence in the patient groups receiving a PA catheter imply that they may have had more CV disease than the control group.

In this study we found that patients 'tuned up' before operation with a PA catheter had fewer adverse intraoperative events ($p < 0.05$), less postoperative cardiac morbidity ($p < 0.05$) and early graft thrombosis ($p < 0.05$), and fewer deaths ($p = 0.08$) than the control group (Table 6). The overall complication rate was 16.2% in the PA catheter groups and 42.9% in the control group. We could find no difference in outcome between the SICU tune-up (group 1) compared to the PIR tune-up (group 2), *i.e.*, the duration of the tune-up was not a significant factor.

More than 36% (36.8%) of our patients with a PA catheter (groups 1 and 2) had normal hemodynamics and LVF without intervention, 26.4% needed fluids alone, and 36.8% needed pharmacologic intervention. Our findings coincide with those of other authors. In a group of 75 patients with PVD, Babu et al.[3] found that 33.3% had normal LVF, 26.7% needed only fluids to improve myocardial function, and 40% needed additional pharmacologic intervention. In 100 high-risk surgical patients, Del Guerico et al.[54] found no patients with normal hemodynamics, 55% needed fluid alone, and 45% needed additional intervention. Interestingly, while Del Guerico et al.[54] found that most of the complications occurred in the last group of patients, we could not confirm this finding. However an implication of this finding is that if the PA catheter is placed immediately before operation, up to 40% of surgical patients may need to have their surgical procedure delayed until optimal hemodynamic status is achieved.

Mangano[61] reviewed studies from the past 35 years of intraoperative predictors of perioperative cardiac morbidity (PCM). He concluded that while both hypotension and tachycardia predict PCM, there are conflicting data about hypertension and insufficient data on arrhythmias. In our study we found more intraoperative episodes of hypotension ($p < 0.05$), tachycardia ($p < 0.05$), and arrhythmias ($p < 0.05$) in the control group (Table 3). While we cannot confirm a causal relationship, many control patients with intraoperative problems did develop postoperative complications.

One of these complications was early (less than 36 hours after operation) graft thrombosis. A high incidence of graft thrombosis in the control group was found on re-exploration not to result from technical errors. We postulate that inadequate blood flow (*i.e.*, CO) may have contributed to this problem and that patients with a PA catheter had less graft thrombosis because of increased perioperative cardiac output.

We found a mortality rate of 1.5% in the patients with a PA catheter compared to 9.5% in the control group, with an overall study mortality rate of 3.4%. It is noteworthy that the only death in the study group followed unexpected exsanguination, an event not influenced by PA catheter monitoring. The two deaths in the control group followed cardiopulmonary arrest. Babu et al.[3] had a 1.3% mortality rate in their study of monitored patients undergoing diverse peripheral vascular procedures. Jamieson et al.[6] found a 1.4% mortality rate in a small subset of unmonitored patients who had femoropopliteal procedures. However only 4.3% of Jamieson's entire study group had diabetes; Babu[3] did not delineate the clinical characteristics of their study group. Diabetes is a significant, independent risk factor for PCM.[61] Seventy-two per cent of the patients in our study had DM, which may account for the higher mortality rate in our control group.

In our study only one patient had a significant complication from insertion of the PA catheter. A small pneumothorax was discovered on CXR after operation in a patient from group 1. The PA catheter had been inserted through the subclavian approach in this patient. Two other patients had non–Q-wave MIs associated with the tune-up. We think this is the first report of morbidity associated with the preoperative tune-up. One patient had no clinical signs or symptoms of MI; had we not drawn cardiac enzymes before and after tune-up we would have assumed it to be an intraoperative complication. We think preoperative MI may be underreported in other clinical studies.

The cost of technology should be included in any benefit analysis. Schoemaker et al.[62] reported that the use of the PA catheter reduced complications, duration of hospitalization, ICU stay, duration of mechanical ventilation, and total cost when used to augment rather than simply normalize circulatory responses in a group of high-risk surgical patients. We found the use of the PA catheter to be cost neutral, i.e., the increased cost of PA monitoring and tune-up in the study groups was offset by the increased cost of treating the complications in the control group. We believe that we failed to find a significant cost difference between groups because of the severity of the underlying vascular disease. These patients commonly require lengthy hospitalization because of the end-organ injury incurred from their disease before the operative intervention.

The insertion of a PA catheter is not therapeutic. While intervention on the basis of catheter-derived information is thought to be beneficial,[63] at least one study refutes this presumption.[64] Why did we find a benefit? It is known that early resuscitation and restoration of oxygen transport improves outcome in trauma patients.[65] Shoemaker et al.[62] have shown that preoperative resuscitation to supranormal cardiovascular indices may be necessary when the postoperative oxygen demands imposed by major surgery are also supranormal. A patient's ability to respond immediately to the oxygen demands imposed by the stress of surgery appears to be one key to reducing postoperative morbidity. In addition we used a highly integrated team approach to the perioperative management of these high-risk patients. A recent study supports the position that a knowledgeable, experienced physician is an essential component of this technology.[66]

The PA catheter, when used by knowledgeable physicians to evaluate and optimize cardiac performance before operation, is cost neutral and improves outcome in patients undergoing peripheral revascularization procedures.

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DISCUSSION

DR. JOSEPH M. CIVETTA (Miami, Florida): I would like to congratulate the authors for performing the first randomized prospective study in the field of invasive hemodynamic monitoring in the 21 years that such devices have been available. Indeed, the lack of data prompted Robin in 1988 to urge a moratorium until efficacy could be established. This paper can be considered a first step to answer such objections.

Furthermore by confining the study to the same surgeons and anesthesiologists, possible treatment bias was controlled. I believe this is important, as an "enlightened team" might choose invasive monitoring because of a better understanding of physiology, and thus could direct therapy more appropriately. In contrast those who do not value monitoring also might select less excellent care, as both decisions would be based on a deficient understanding of physiology.

This study adds to the scientific basis for invasive monitoring of elective surgical patients, just as fiscal motivations by third-party payers for exclusion of the same patients is mounting.

There are two areas that need further analysis and investigation, however. First limb salvage surgery seems to be a limited physiologic stressor, thus minimizing the need for or impact of preoperative tuning. Major abdominal vascular procedures might be a more important and more appropriate focus.

Second only 10 of 89 patients had complications. As a result, there was insufficient impact on resource usage to show differences.

Zook and Moore, and later Drucker, have shown that complications cost money, and, I might add, far more than ever could be saved by exclusion of these patients from the intensive care unit.

Perhaps there is a type II or beta error. There would be a significant difference if a larger number of patients at known higher risk were studied. I would urge the authors to continue the study, as financial arguments would carry the most weight with third-party payers.

I have one question. Could you quantify the amount of tune-up—that is, blood, colloid, and vasodilators—that were used in the two groups? For unless there was a significant quantity required, major outcome differences are unlikely.

DR. LOUIS R. M. DEL GUERCIO (Valhalla, New York): I have been anxiously awaiting this excellent controlled trial from the University of Minnesota. I have long been an advocate of preoperative invasive monitoring. But in a sense I was hoisted on my own petard, because I made such a fuss for it at our own institution that when we applied for a controlled trial the community review board said that it would be unethical because we were so committed to the concept.

I agree that this is an excellent, well-controlled study and most important. As a matter of fact, I had submitted a competing abstract to this society, and I agree with the Program Committee that this paper is much better than the one we submitted.

What we had shown in some 300 patients, all uncontrolled, was that when one did multivariant analysis of these patients, there was no correlation whatsoever of age with length of stay, death, or complications. In other words, age bias is eliminated by proper preoperative monitoring and optimization of patients. And those were 300 patients aged 60 to 93.

We were surprised to find, however, that the mortality rate and complications did correlate with a decreased hematocrit, with a mild metabolic alkalosis, and a body mass index that was slightly below normal. Now these are all constitutional factors, which probably are related to the patient's chronic disease and we are certainly not saying that improvement of these factors, such as giving blood or parenteral nutrition, would change the outcome.

When you peel off the layer of the onion of the most frequent complications in the high-risk elderly patient, you then find that constitutional factors are associated with death. But the most important thing that we found was that no longer should one be biased in planning surgery of the aged, as long as you are willing to do preoperative monitoring.

The question I would like to ask the Minnesota group is, because you have shown that this is statistically important, has it changed the approach at the University of Minnesota hospitals to the high-risk patients? In other words, outside of this study, are your clinicians using preoperative invasive monitoring?

DR. CHARLES E. LUCAS (Detroit, Michigan): Your beautifully presented paper leads one to suspect that your conclusions are valid. I am worried about your control patients, however, as to what are the end points that the anesthesiologist used for their resuscitation, because they no longer had central pressure measurements. Were there differences in urine output in the two groups? Was there some other reason to explain the increased incidence of low blood pressures in operations that do not involve a deep body cavity and should not be associated with hypotension?

DR. DONALD J. GLOTZER (Boston, Massachusetts): I just wanted to know if you thought that the superior results in the "tune-up" patients were due to the "tune-up" or due to the fact that the anesthesiologists had the Swan-Ganz catheters in place to use intraoperatively?

DR. MALCOLM O. PERRY (Nashville, Tennessee): It is unclear to me exactly what was deleted in the control group. It appears that the predominant difference was in the evaluation of the Starling curve. Surely in those patients in the control group, if there were nutritional, fluid, or hemodynamic abnormalities, these also were corrected before surgery. If not the study would have been biased from the beginning.

There appears to be an incidence of 20% of early graft thrombosis in the control group. I would appreciate it if the authors would comment on these two items.

DR. ROBERT J. CORRY (Iowa City, Iowa): A very nice paper by the University of Minnesota. I wonder if you would take it one step further in these patients and evaluate their hearts with IVGs and even coronary angiograms if they are in such a high-risk category, which some of us do, and I believe you do with diabetics awaiting a double-organ transplant.

In this way you might ferret out the patients who might have perioperative coronary problems.

DR. J. F. BERLAUK (Closing discussion): First I would like to answer the question about the PA catheter and whether the difference between the study groups and the control group could be attributed to intraoperative monitoring with a PA catheter in the former groups. After all better intraoperative monitoring might be expected to improve outcome in high-risk patients. Dr. Schoemaker has published a study in the December 1988 issue of *Chest*, however, and he found that if the PA catheter was only used to monitor, but not to augment, the cardiac output in his study group of patients, their outcome was no different from that of patients receiving central venous pressure monitoring. So although you cannot tell from our study that the outcome difference was based on the tune-up alone, when you combine this information, I think it indicates that the tune-up was more significant than the fact that the PA catheter was also available for monitoring.

We chose this group of patients not only because they had a high perioperative risk of morbidity and mortality, but also because they were undergoing a relatively low-risk procedure, distal limb salvage surgery. In other words, the stress of the surgical procedure played a relatively minor role in outcome. Therefore we thought we had the perfect group of patients in which to test whether the PA catheter and the tune-up really made a difference in outcome. Although we would agree that you could use the tune-up in a group of patients with higher operative risk and probably derive more benefit, we have not done that yet, but plan to do so in the near future.

That leads to the question, "do the other surgeons at the university endorse this approach to some of their high-risk patients?" I think that

I can say that we have enthusiastic endorsement for this approach. The preoperative tune-up has carried over into other groups of patients, to whom we have not really proven a benefit, such as extensive gynecologic and cancer surgery patients, who have risk factors other than just cardiovascular disease. In addition some of the patients undergoing major vascular surgery, intra-abdominal, intrathoracic, or other high-risk surgical procedures now receive this approach at the University Hospital.

Dr. Civetta asked whether we could quantify total blood and total fluids that were used during the study. Those data are available but we did not look at them. Every time we made an intervention in a study patient, we recorded which drugs were used, and how much and what kind of fluid was given. That is all on our database. It is available, and we will look at it now that the question has come up.

The intraoperative end points that we used in all patients followed a protocol. Anesthesia for almost all of the study was administered by one or two anesthesiologists, who were instructed to act on any reduction in blood pressure or increase in heart rate that was approaching the levels that I had outlined as the limits of the adverse intraoperative events. We did not wait until those events occurred and then simply record them. Anesthesiologists were instructed to intervene in whatever manner was appropriate to try to correct those adverse events before they reached significance.

The incidence of graft thrombosis was high in the control group, and that was a striking finding that we did not expect. All of those patients had graft thromboses within 36 hours of their operation, and on surgical re-exploration, no technical problem with the original anastomosis could be identified. We ascribed all these thromboses to inadequate cardiac output or inadequate flow.

Changing Trends in Necrotizing Enterocolitis

Experience With 302 Cases in Two Decades

JAY L. GROSFELD, M.D., HENRY CHEU, M.D., MARC SCHLATTER, M.D., KAREN W. WEST, M.D.,
and FREDERICK J. RESCORLA, M.D.

From the Section of Pediatric Surgery, Department of Surgery, Indiana University School of Medicine and the James Whitcomb Riley Hospital for Children, Indianapolis, Indiana

Three hundred two infants with necrotizing enterocolitis (NEC) were treated from 1972 to 1990. One hundred eighteen were treated medically while 184 infants required operation. Comparisons were made between two treatment periods, 1972 to 1982 (n = 176) and 1983 to 1990 (n = 126). Infants in the more recent era were of lower birth weight (1505 ± 853 g *versus* 1645 ± 836 g), earlier gestational age (30.4 ± 4.7 weeks *versus* 32.4.5 weeks; [$p < 0.01$]), had symptom onset at an older age (15.7 ± 13.9 days *versus* 10.0 ± 10.8 days; [$p < 0.001$]), and a lower incidence of hyaline membrane disease ($p < 0.001$). Fewer patients in the 1983 to 1990 group had acidosis ($p < 0.001$) and severe oliguria ($p < 0.001$). Operation was performed sooner after diagnosis in the second group (2.6 *versus* 3.8 days; [$p < 0.001$]). Survival was unaffected by sex, maternal complications, or whether infants were inborn or transferred from other facilities. Improved survival (1983 to 1990) was observed in those infants between 24 to 27 weeks gestation ($p < 0.002$) and those weighing less than 1000 g ($p < 0.001$). Since 1983 portal vein air (PVA) on abdominal radiographs was used as an indicator for operation. Survival in infants with PVA has improved from 29% to 64% ($p < 0.02$). Despite patients being more immature and weighing less, the overall survival rate improved from 58% (1972 to 1982) to 82% (1983 to 1990) ($p < 0.001$). Operative survival rate improved from 51% to 75% ($p < 0.002$). Long-term survival was 75% overall and 65% for surgical infants in the 1983 to 1990 group ($p < 0.05$).

NECROTIZING ENTEROCOLITIS (NEC) IS a life-threatening intra-abdominal condition affecting 1% to 2% of admissions to most newborn intensive care facilities.[1-4] Most cases occur in premature and low-birth-weight infants who often are already compromised by other associated illness. The purpose of this report is to describe changing trends noted in infants with NEC treated at a single neonatal intensive care facility in the past two decades, emphasizing those factors that have resulted in a significantly improved survival rate.

Patients

This report concerns 302 infants with NEC treated at the James Whitcomb Riley Hospital for Children at the Indiana University Medical Center campus between the years 1972 to 1990. One-hundred eighteen infants were managed medically while 184 required operative intervention. Evaluation and comparisons were made between two treatment periods, 1972 to 1982 (n = 176) and 1983 to 1990 (n = 126). The Student's t test and the Yates corrected chi-square analysis were used to evaluate statistical validity. A probability value of less than 0.05 was considered significant. Fifty-four of one hundred seventy-six infants in the 1972 to 1983 era and 30 of 126 cases in the 1983 to 1990 group were born locally on the Indiana University Medical Center campus. Seventy per cent of the patients in the 1972 to 1982 group and 77% of the infants in the 1983 to 1990 group were transferred to our facility from other institutions. The admitting diagnosis was NEC in 66% of the patients in the early group and 68% of infants in the latter group. The remaining infants were admitted for other complications of prematurity and developed NEC after admission. Boys (n = 158) and girls (n = 144) were similarly affected in both groups and there was no significant difference in occurrence or outcome relating to the patient's sex. Infants in the more recent era were of lower birth weight (1505 ± 853 g *versus* 1645 ± 836 g), of earlier gestational age (30.4 ± 4.7 weeks [range, 23 to 40 weeks] *versus* 32.4 ± 4.5 weeks [range,

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Address reprint requests to Jay L. Grosfeld, M.D., Chairman, Department of Surgery, Indiana University Medical Center, 702 Barnhill Drive—Riley K-21, Indianapolis, IN 46202-5200.

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24 to 41 weeks]; $p < 0.01$), and had symptom onset at a later age (15.7 ± 13.9 days *versus* 10.0 ± 10.8 days) than in the 1972 to 1983 group ($p < 0.01$). More than 90% of patients had received enteral intake before the development of symptoms in both groups, including 6% receiving breast milk. A few patients (7% in each group) developed NEC without receiving enteral feedings. Maternal age, complications of pregnancy, incidence of multiple births, resuscitation at birth, and the need for ventilator support were similar in both groups. The incidence of congenital heart disease was similar in both groups; however less hyaline membrane disease was noted in the 1983 to 1990 era (70% *versus* 36%; $p < 0.001$) (Table 1). There was no significant difference in the incidence of patent ductus arteriosus in the two groups. Lethargy and increased gastric residuals following gavage feedings were the earliest signs of a potential clinical problem in most cases. The most frequent clinical findings included abdominal distention in 172 infants, abdominal tenderness in 128, acidosis in 118, blood in the stool in 107, bilious gavage aspirate or vomiting in 88, abdominal wall erythema in 67, and oliguria in 47 cases. The presence of a right-sided abdominal mass was seen in just a few infants in both study groups. Bilious vomiting, abdominal tenderness and distension, and abdominal wall erythema were less commonly observed in the 1983 to 1990 study group (Table 2). The incidence of blood in the stool was similar. Severe acidosis (85%) and oliguria (39%) were more commonly noted in the earlier decade. In contrast 39% of infants managed from 1983 to 1990 had severe acidosis ($p < 0.001$) and only 9% had significant oliguria ($p < 0.001$) (Table 2). The diagnosis of NEC was confirmed by evaluation of erect and recumbent abdominal radiographs. Pneumatosis intestinalis, pneumoperitoneum, and the presence of portal vein air was observed in a similar number of cases in each study group (Table 3). Fewer patients had fixed dilated bowel loops in the 1983 to 1990 era. Infants were initially managed with orogastric suction, vigorous fluid resuscitation, including the use of crystalloid, colloid, platelets, and blood transfusion as necessary. Parenteral antibiotics were administered in all cases. Patients were frequently re-evaluated with serial examinations, abdominal radiographs, pH and blood gas monitoring, and platelet counts. While a diminished platelet count was often observed, the presence of thrombocytopenia alone neither indicated the need for operation nor determined outcome. Indications for operation included pneumoperitoneum, progressive clinical deterioration, or other evidence of peritonitis or perforation in patients in whom medical therapy failed. Paracentesis was used in two patients in each group with ascites to determine if bacteria or fecal content was present in the peritoneal cavity. In the second decade operative intervention was performed sooner after initial diagnosis of NEC than in the earlier group (2.6 *versus* 3.8 days; $p < 0.05$). At operation perforation was noted in 66 of 101 infants in the earlier group and 50 of 83 patients required operation between 1983 and 1990. The difference was not significant. Seventy-five per cent of the patients in the first group had involvement of the terminal ileum and right colon as compared to 61.4% of cases in the second group (p

TABLE 1. *Necrotizing Enterocolitis: Patient Data*

Findings	1972–1982	1983–1990
Birth weight	1645 ± 836	1505 ± 853
Range (g)	680–3693	470–3970
Gestational age*	32.4 ± 4.5	30.4 ± 4.7
Range (weeks)	24-41	23–40
HMD†	73%	30%
CHD	n = 43	n = 38
Age at diagnosis*	10 ± 10.8	15.7 ± 13.9
Range (days)	(0.5–55)	(0.5–92)
Interval to surgery‡	3.8 days	2.6 days

* $p < 0.01$; †$p < 0.001$; ‡$p < 0.05$.

The infants in the second study group (1983–1990) were characterized by a lower birth weight, earlier gestational age, had less hyaline membrane disease and underwent operation sooner after diagnosis.

TABLE 2. *Necrotizing Enterocolitis: Clinical Findings*

	1972–1982	1983–1990	
Observations	No. (%)	No. (%)	p value
Abdominal distention	99 (98)	73 (86)	$p < 0.01$
Acidosis	85 (84)	33 (39)	$p < 0.001$
Abdominal tenderness	77 (76)	51 (61)	$p < 0.04$
Bilious aspirate	64 (65)	24 (28)	$p < 0.001$
Erythema	47 (46)	20 (24)	$p < 0.004$
Oliguria	40 (39)	7 (9)	$p < 0.001$
Bloody stool	59 (58)	38 (48)	p = NS

An evaluation of the clinical findings indicated that acidosis, oliguria, and abdominal wall erythema (all signs of advanced disease) were noted less frequently in the 1983–1990 study group.

TABLE 3. *Necrotizing Enterocolitis: Radiologic Findings*

	1972–1982	1983–1990	
Observation	No. (%)	No. (%)	p value
Pneumatosis	77 (76)	57 (67)	p = NS
Fixed dilated bowel loops	76 (75)	36 (43)	$p < 0.001$
Free air	46 (46)	34 (41)	p = NS
Portal vein air	35 (35)	22 (26)	p = NS

The radiographic findings were similar between the two study periods except for fixed dilated bowel loops which were less frequently observed between 1983–1990.

= NS). Of interest was the fact that fewer infants in the second group had NEC totalis. Extensive bowel necrosis was observed at the time of the initial operation in 38 infants (38%) in the 1972 to 1982 group and in 12 infants (14.4%) treated from 1983 to 1990 ($p < 0.02$) (Fig. 1). In patients with perforation and/or localized ischemic disease, bowel resection and temporary enterostomy was the treatment of choice. In infants with near-total bowel necrosis, the abdomen was simply closed without resection. Second-look procedures were attempted in six cases and in only two was a limited resection possible. Peritoneal cultures obtained at the time of operation grew a wide variety of bacterial organisms, including *Escherichia coli, Klebsiella pneumonia, Staphylococcus epidermidis, Bacteroides fragilis.* and *Clostridium perfringens.* There was no single organism that predominated in either of the two study periods. After operation most infants were maintained on ventilator support, orogastric decompression, antibiotics, and bowel rest. Almost all of the infants received total parenteral nutritional. Feedings were withheld for 7 to 10 days. Despite the fact that patients in the more recent era were smaller and more fragile, an improved survival was observed. In the 1983 to 1990 group, survival was improved in infants who weighed less than 1000 g ($p < 0.01$) and those who were between 24 to 27 weeks gestational age ($p < 0.002$) (Figs. 2 and 3). Survival among medically treated patients from 1972 to 1982 was 68% (51 of 75) *versus* 98% (42 of 43) between 1983 and 1990 ($p < 0.001$). For those undergoing surgical intervention, 51 of 101 infants survived (51%) between 1972 and 1982 whereas 62 of 83 (75%) surgically managed infants in the 1983 to 1990 era survived ($p < 0.002$) (Fig. 4). These data represent 30-day postoperative survival rates for those undergoing a surgical procedure and 30-day survival rates from the time of diagnosis in medically treated infants. The overall survival rate for the entire group was 58% (102 of 175) in the 1972 to 1982 decade compared to 82% (104 of 126) in the more recent era ($p < 0.001$). Of interest is the fact that from 1972 to 1982 infants for whom portal vein air was demonstrated on plain abdominal radiographs had a 71% mortality rate; this finding was not considered an indication for operative intervention. From 1983 to 1990, however, portal vein air was considered a relative indication for operation resulting in earlier surgical

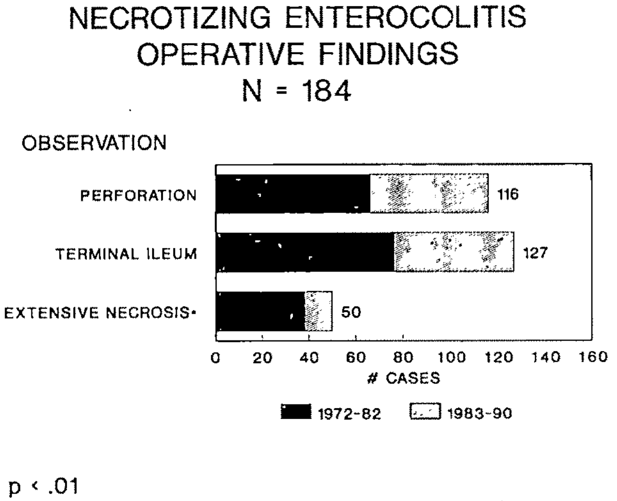

FIG. 1. This bar graph indicates the findings at operation in 184 infants with NEC. The incidence of perforation and involvement of the terminal ileum and right colon were similar between the two groups. However extensive bowel necrosis was observed less frequently in the 1983–1990 study group ($p < 0.01$).

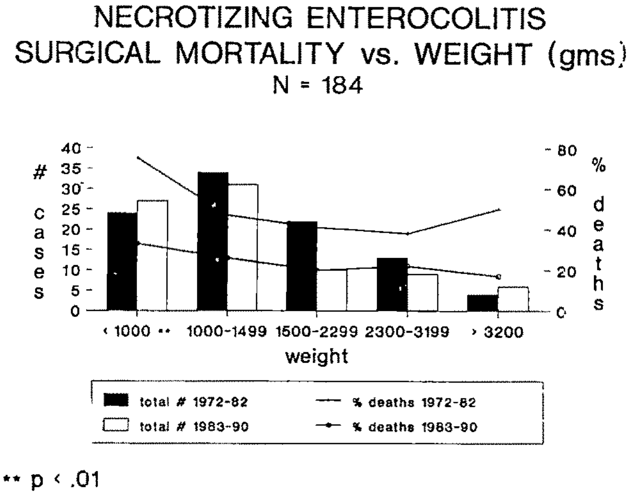

FIG. 2. This composite bar and line graph compares the number of cases (bars) and deaths (lines) in each weight group. The mortality rate was lower in the 1983–1990 study group and reached significance in infants weighing less than 1000 g ($p < 0.01$). There were too few infants weighing more than 3200 g to analyze.

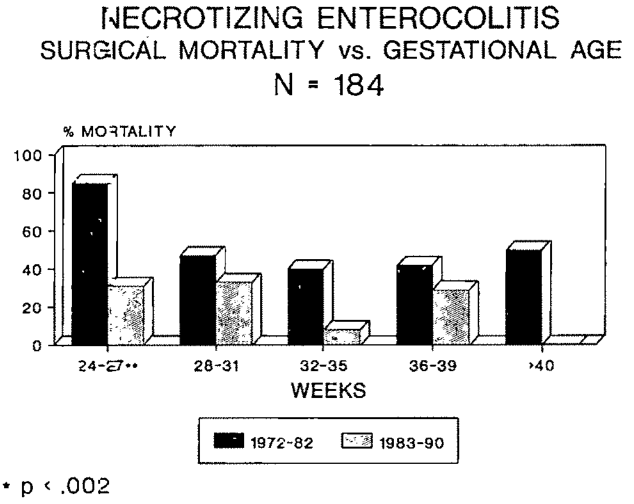

FIG. 3. This bar graph evaluates death and gestational age. The mortality rate was lower for each age group in the 1983–1990 era. The data were highly significant for the youngest age group (24–27 weeks) ($p < 0.002$).

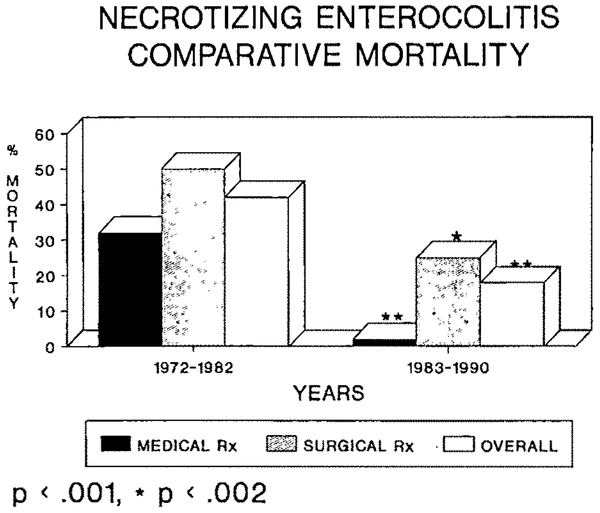

FIG. 4. This bar graph demonstrates significantly improved medical, surgical, and overall mortality rates in the 1983–1990 study group. The overall survival at the present time is 82% (75% for surgically treated infants).

intervention and an improved survival rate. In the 1972 to 1982 study group, 5 of 10 patients with portal vein air treated medically died and had evidence of missed bowel necrosis at autopsy. Twenty-five of thirty-five infants with portal vein air and delayed surgical referral who eventually underwent operation also died. In each instance extensive bowel necrosis was identified. In the more recent era, 3 of 25 patients with portal vein air also were treated medically. In each of these instances the patients were clinically stable, had no early complications, and all survived. One of these infants subsequently developed intestinal obstruction and at laparotomy a walled-off perforation and abscess was noted. From 1983 to 1990, 14 of 22 infants with portal vein air and other evidence of instability who were managed with early surgical intervention survived, reducing the mortality rate in these selected cases to 36% ($p < 0.02$) (Fig. 5). Six of the eight infants who died had extensive bowel necrosis. The occurrence of late deaths (beyond the 30-day observation period) was similar in both study groups; however the long-term overall survival rate in the most recent decade continues to exceed 75% (65% in surgical patients) as compared to 49% for all patients in both the medically and surgically treated groups in the earlier decade ($p < 0.05$). No statistical comparisons were made between the medical and surgical patients because the surgical cases represented a skewed group of infants with more severe disease in whom medical therapy failed. We think these data imply that earlier diagnosis and referral, prompt resuscitation, advances in sophisticated neonatal intensive care, and expeditious operation, when indicated (including patients with portal vein air), has improved the survival rate in infants with NEC.

Discussion

The exact etiology of NEC is unknown. Necrotizing enterocolitis has become a major clinical problem during the past 25 years as a result of improvements in modern neonatal intensive care and increased survival of the premature and low-birth-weight infant. The relatively common survival of premature infants weighing less than 1000 g in the current era further emphasizes this fact. Ninety per cent of afflicted infants are premature, while approximately 10% are full term.[1,5] Although the pathogenesis of NEC remains somewhat obscure, some risk factors have been identified and a number of theories proposed.[6–10] Predisposing factors include intestinal ischemia, the presence of intraluminal nutrient (formula), and the intestinal bacterial flora.[6,8,10] The concept of an hypoxic or hemodynamic insult resulting in splanchnic vasocontriction and reduced mesenteric flow inducing bowel mucosal hypoxia and rendering the intestine susceptible to injury was long considered the most probable cause.[7] The fact that many premature infants with serious illnesses, including respiratory distress syndrome, birth asphyxia, congenital heart disease with heart failure, sepsis, shock, hypothermia, and polycythemia/hyperviscosity syndrome developed NEC supported this concept.[1,4] However, in a significant number of cases, none of these predisposing problems could be identified. Iatrogenic factors associated with an increased incidence of NEC include the use of umbilical artery catheters, exchange transfusion, and the administration of certain medications, including indomethacin, aminophylline, and vitamin E for the treatment of patent ductus arteriosus, apneic episodes, and for prevention of retrolental fibroplasia, respectively.[11–15] Other studies have

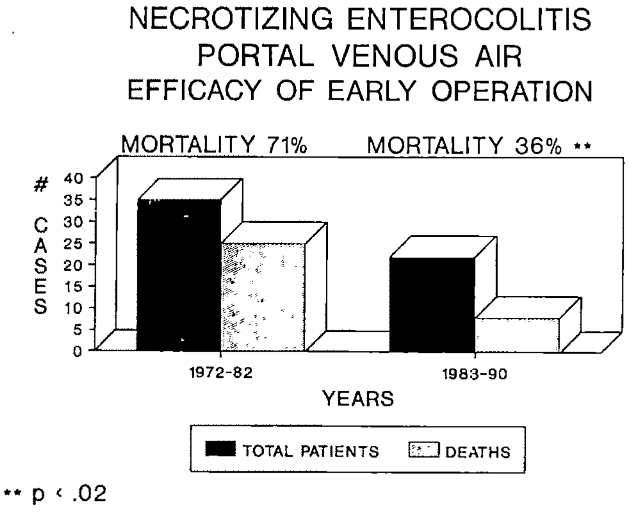

FIG. 5. This bar graph demonstrates a significantly lower mortality rate in infants with portal vein air on abdominal radiographs in the 1983–1990 study period ($p < 0.02$) when this finding was considered an indication for early operation.

documented the occurrence of NEC following intestinal rotavirus infection and in association with maternal cocaine addiction.[16–18] Other factors, including intestinal mucosal immaturity, mucosal absorption of macromolecules, decreased humoral immunity characterized by diminished levels of lamina propria IgA and circulating IgM, and varying premature feeding practices and formulas also have been implicated.[4,9,19–21] More recently evidence that release of cytotoxic free radical anions such as superoxide, certain cytokines (tumor necrosis factor alpha), interleukins [IL-1]), platelet-activating factor, bacterial endotoxins (lipopolysaccharide), and bacterial translocation from the bowel lumen also may play a role in the progression of the disease process once an injury to the mucosa occurs.[9,22,23] All of these observations suggest that NEC is a complex process that occurs predominantly in the very low–birth-weight premature infant (a susceptible host) with potentially compromised immature intestinal mucosa and decreased systemic immune function. The mucosal injury can be initiated by a variety of clinical factors (including hypoxia) and in the presence of a potentially invasive intestinal flora sets forth a series of events that includes a breakdown of the mucosal defense barrier and the elaboration of cytotoxic inflammatory mediators and other substances that result in bowel necrosis.[9,23,24]

The earliest clinical observation that raises the index of suspicion regarding the occurrence of NEC in the newborn nursery or newborn intensive care unit (NICU) setting is increased gastric residuals when attempting gavage feedings. Premature infants have an immature swallowing reflex and require orogastric gavage feeding techniques. The NICU nurse is often the first health professional to recognize that a potential problem is at hand. Laboratory data and abdominal radiographs are obtained to confirm these concerns. Controversy exists regarding the effectiveness of early feedings in high-risk infants. While there are proponents of both active early feeding *versus* no feedings for up to 10 days, the results of these practices are inconclusive.[20,21] Ninety-three per cent of the patients in this study were fed before the onset of NEC, suggesting that intraluminal nutrient may act as a substrate for bacterial overgrowth and promote invasiveness in the presence of a mucosal injury. However NEC developed in 7% of infants in both study groups that were never fed. Cikrit et al.[11] reported a number of risk factors associated with a significantly increased mortality rate in infants with NEC, including acidosis, oliguria, umbilical artery catheters, birth weight less than 1500 g, gestational age less than 27 weeks, extensive bowel necrosis sometimes affecting almost all the intestine (NEC-totalis), and the presence of portal vein air on abdominal radiographs. These factors were much more commonly observed in patients in the 1972 to 1982 group. In the 1983 to 1990 era, fewer infants developed significant acidosis or oliguria, suggesting that supportive therapy was initiated earlier and probably played a role in the improved survival observed in this second group of patients. The fact that fewer infants (14.4% *versus* 38%) had extensive bowel necrosis in the latter era also supports the impression that earlier recognition and more prompt interventional therapy limited progression of the disease process. Improved survival was also observed in the medically treated patients in the 1983 to 1990 group. The time interval from diagnosis to surgical intervention was significantly less in the 1983 to 1990 study group, indicating a more expeditious transfer of infants that failed medical therapy to the pediatric surgical service. Sometimes, however, infants with NEC present with sudden hemodynamic collapse and a rapid fulminant decline within a few hours of the onset of illness, documenting the spectrum of disease that may be encountered.[8]

The indications for operation in infants with NEC vary somewhat from institution to institution. Most surgeons agree that pneumoperitoneum is an absolute indication for operation.[25–29] Failure to respond to medical management (following adequate fluid resuscitation, gastric decompression, and the administration of broad-spectrum antibiotics) is characterized by evidence of continued clinical deterioration, including hemodynamic instability, abdominal distension and tenderness, progressive thrombocytopenia, and metabolic acidosis. These findings are consistent with intestinal ischemia and are certainly adequate reasons to proceed with laparotomy, ideally before the occurrence of perforation and widespread peritonitis. Although the presence of portal vein air has long been recognized as a radiographic finding in infants with NEC, until recently few investigators considered this an indication for operation.[30,31] In the infants treated from 1972 to 1982, portal vein air was associated with an increased incidence of extensive bowel necrosis (38%) and a high mortality rate (71%). It seemed apparent that infants with portal vein air often had advanced bowel ischemia. From 1983 to 1990 portal vein air was considered a relative indication for early operation. Early intervention was associated with a decreased mortality rate (36%). In cases of NEC with either perforation or gangrenous bowel, the procedure of choice is resection and exteriorization of the proximal and distal bowel.[11,26,27] In instances of colonic involvement, proximal enterostomy or colostomy and a distal Hartmann pouch has been an effective procedure in our hands. A few surgeons in both the United States and Australia are advocates of bowel resection and primary anastomosis in selected cases of NEC.[32,33] We have not attempted primary anastomosis in these cases and consider exteriorization and early delayed anastomosis a safer approach to surgical management in the seriously ill premature infant. In instances in which most of the bowel is ischemic or necrotic (NEC totalis), the mortality rate is more than 90%. High diverting jejunostomy has been advocated but we have not been impressed with the results of this procedure.[34] Although we have usually closed the abdomen and performed a second-look laparotomy after 24 to 48 hours if the infant survives supportive measures (as suggested by others), only two of six patients had any improvement in the appearance of the bowel allowing resection.[35] Four of the patients died and the two surviving infants had short bowel syndrome. In the current era, NEC is the leading cause of short bowel syndrome in infancy.[36] Ein et al.[37] inserted a right lower quadrant drain through a stab wound under local anesthesia in the NICU as a preliminary form of treatment in unstable premature infants with bowel perforation. Drainage relieved increased intra-abdominal pressure, improved ventilation, drained contaminated material, permitted resuscitation, and allowed stabilization of the infant in preparation for delayed laparotomy. In some instances no further treatment was necessary. Survival was similar to those infants undergoing immediate laparotomy. Cheu et al.[38] reported that primary peritoneal drainage was a useful adjunct to resuscitation of infants with NEC and perforation who weighed less than 1000 g but was not an alternative to laparotomy. While we have no personal experience with this technique, we believe it is a procedure that is worthwhile considering in a select group of infants with NEC.

Major postoperative complications include fluid and electrolyte disturbances, malabsorption and feeding difficulties due to excessive gastrointestinal tract losses from the stoma of a shortened bowel, cholestatic liver disease related to the use of total parenteral nutrition, and bacterial and fungal sepsis.[11,30,39] Because the presence of a stoma can be a problem, we recommend early closure when the infant has stabilized and demonstrated a growth response on enteral drip feedings supported by parenteral nutrition. Before attempted enterostomy closure, a contrast study of the distal bowel should be obtained to document the presence of a stricture, which may occur in 10% to 20% of cases. Strictures are more commonly located in the colon and may also occur after successful medical treatment.[29]

The mortality rate for infants with NEC weighing less than 1000 g and whose gestational age is 24 to 27 weeks was especially high during the years 1972 to 1982. In addition to early operation and resection of necrotic bowel, improvements in neonatal intensive care (surfactant therapy, jet ventilators, and so on) in the 1983 to 1990 era has resulted in a significant improvement in survival of these very low-birth-weight infants. Although the improvement in survival that has been achieved during the 1983 to 1990 treatment period has been gratifying, the morbidity and mortality rates related to NEC continue to be significant. As more premature infants survive their arrival in the extrauterine environment, NEC will continue to be a major clinical problem.[40] Prevention of the occurrence of NEC is a high priority, with reduction of the number of premature births an obvious goal but one that has been difficult to achieve. In a prospective study from Austria, Eibl and associates[19] reported that an oral immunoglobulin preparation containing 73% IgA and 26% IgG may protect high-risk premature infants from developing NEC. Bauer et al.,[41] reported a decreased incidence of NEC in infants born to mothers receiving prenatal corticosteroids in an attempt to stimulate lung maturation.. They theorized that steroids caused maturation of the immature intestinal mucosa as well as the lung. More recently in a controlled prospective trial from Argentina concerning 466 pregnant women with premature labor, Halac et al.,[42] reported that prenatal administration of steroids significantly reduced the incidence of NEC. Administration of postnatal dexamethasone to neonates for 7 days did not decrease the incidence of NEC as effectively as prenatal treatment but improved the clinical outcome of the infants with NEC when compared to untreated controls. Both of these fascinating studies strongly suggest that an immature intestinal mucosal barrier plays a significant role in the pathogenesis of this disorder. Further prospective studies to develop the most appropriate feeding schedule, evaluate prenatal steroid administration, the effect of enteral immunoglobulin administration, the possible protective effect of glutamine (or other mucosal protective substances), and the search for a potential marker for intestinal mucosal maturity may refine the future management of premature infants at risk for NEC.

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DISCUSSION

DR. JAMES A. O'NEILL, JR. (Philadelphia, Pennsylvania): On the basis of reading this manuscript, I would like to make the following comments and ask the following questions. This disease is associated with a variety of risk factors, as Dr. Grosfeld has pointed out. The differences in one or two of these over the two periods might not really be expected to have influenced the pattern of presentation very much, and that is actually what he saw.

Looking at your data, in other words, Dr. Grosfeld, the pathogenesis of this disease has not appeared to have changed.

In a recent report of 150 surgical patients by our group, we noted similar changes in terms of later onset of disease, and clinical consequences of the disease, such as acidosis and oliguria, probably related to better supportive care, as you pointed out. We also agree that portal venous gas is a sign of advanced disease and is a relatively strong indication for operative intervention.

Most series, including our own, report a ratio of 70% to 80% medically or supportively treated patients and 20% to 30% surgical treatment. Conversely you've presented a ratio of 40% medical to 60% surgical. Does this mean you are more aggressive with surgical intervention, or is your series somehow reflecting selection, or is it a different pattern of presentation?

Although you operated earlier, about 24 hours earlier than you had in the first period, you encountered the same incidence of perforation, something of a paradox, yet you had far fewer cases of total intestinal gangrene. Was this due to a changing pattern of disease or earlier operation? I'm not sure whether it is possible to answer that from the data.

May I have that single slide, please? We also have seen the same pattern of improvement in survival as Dr. Grosfeld, except in one disturbing respect: maternal cocaine abuse. Cocaine is known to have a primary alpha-adrenergic effect, which decreases uterine blood flow, increases fetal mesenteric vasoconstriction, and thus produces fetal hypoxia, previously presumed risk factors.

These figures shown here demonstrate the severity of this in a recent study of 61 patients with necrotizing enterocolitis by our group, just reported this month. We therefore are very aggressive in our readiness to operate on such patients.

Also in an analysis using a statistical tool called "matched odds ratio," you can see that there is a two-and-a-half times chance of developing a necrotizing enterocolitis if there has been intrauterine exposure to cocaine. It further confirms the ischemic nature of this disease and shows a changing pattern.

Finally let me say that you presented a great deal of worthwhile data that will be useful to those who are trying to understand and treat this very difficult population of patients. Thank you very much.

DR. MARC I. ROWE (Pittsburgh, Pennsylvania): This is the largest series of very small infants with necrotizing enterocolitis (NEC) and has the highest survival rate.

The whole problem of pathogenesis of NEC is interesting. If you look carefully at the matched control studies, the various perinatal factors, such as exchange transfusions, shock, and hypoxia, are the same in the match control groups and the NEC groups. Almost all of these so-called pathogenic factors occur during the first few days of life; yet, as is very clearly shown by Dr. Grosfeld's data, NEC takes 15 days to develop. If the perinatal factors, such as intrauterine hypoxia, are related to the pathogenesis of NEC, why does it take 2 weeks after the insult for NEC? It seems these factors, rather than being associated with pathogenesis, delineate a population at risk.

The current theory of pathogenesis involves two factors, bacterial colonization and immaturity. The patients are usually premature ICU babies. Their GI tract becomes colonized with potential pathogens and, because of prematurity, they have an immature gut barrier. Bacterial translocation and invasion develops. The inflammatory responses of endotoxins, cytokines, and other humoral and cellular byproducts cause further bowel damage and the clinical picture of NEC.

Both Dr. O'Neil and Dr. Grosfeld mentioned portal vein gas as a secondary indication for operation, not a primary indication; yet Dr. Grosfeld's data are striking. Patients that had portal vein gas were all operated on and had dead gut. We looked at 168 patients with necrotizing enterocolitis, 24 patients had portal vein air; 92% were operated on, and 100% of that group had necrotic gut, usually very extensive. The average time from portal vein gas detection to operation was over 24 hours. This suggests that if you see portal vein gas, you are going to see dead gut and you had better operate now, rather than wait 24 hours or longer. I would be interested in the interval between portal vein gas and operation in Dr. Grosfeld's patients.

Because historical controls were used in this study, I am concerned that the change in survival might be due to the fact that the disease itself changed, rather than that diagnosis and treatment improved. The preoperative perforation rate was 46% in the historical group of patients, and 41% in the new group, essentially the same. If the improved results were due to early diagnosis, as suggested by the authors, why was the incidence of perforation the same in both groups? It should have been less with earlier diagnosis.

I enjoyed the paper very much. Thank you very much.

SECRETARY GEORGE SHELDON (Chapel Hill, North Carolina): Immunoglobulin A (IgA) is the antiseptic paint of the gut, and it is one of the last proteins to decrease in undernutrition, stress, sepsis, etc., which may bear on Dr. Rowe's question of why does this take 12 to 14 days, which is the interval that it takes this protein level to start to decrease.

Because about 80% of the IgA comes through the bile in the form of secretory biliary IgA, it would seem an easy experiment to do to measure bile obtained intraoperatively. Have you done that?

DR. DAVID TAPPER (Seattle, Washington): Jay, your laboratory has at times evaluated superoxide radicals and oxygen scavengers, and in light of some of the new work on bacterial translocation and the fact that we do not have a model for necrotizing enterocolitis (NEC), I wonder what your feeling might be about using medications in patients who are being medically managed with NEC. Specifically can allopurinol or some of the monoclonal antibodies that we heard about for anti-inflammatory or anti-TNF (tumor necrosis factor) be given to patients who are undergoing NEC watch?

DR. JAY L. GROSFELD (Closing discussion): I appreciate the comments of our discussants. With regard to both Dr. O'Neill's and Dr. Rowe's concern about the similar numbers of cases of perforation in both study groups; perforation can happen quite early in the course and may be a beneficial occurrence, because there is no question about treatment. The infant has a prompt operation. In our experience the patients who had perforation actually had the best survival. The major difference in the more recent era was that surgery was performed earlier on the other patients not responding to medical therapy, in whom the indications for surgery perhaps are not as clear.

The fact that we had a 60% surgical intervention rate is due to the fact that our facility is a tertiary children's referral hospital. Seventy to seventy-seven percent of the NEC patients came from other facilities and represent a skewed group of higher risk patients.

With regard to portal vein air, Dr. Rowe asked why we did not operate on all the patients. In the 1972–82 study group, there were 10 babies with portal vein air that were managed medically. Five died and had necrotic bowel that was unsuspected by our medical colleagues, yet five lived and did fine. In the 1983–90 group, three relatively stable babies with portal vein air were also managed medically. They all did well, although one infant subsequently developed a bowel obstruction. Despite the fact that most babies with portal vein air have advanced disease, not every patient has necrotic bowel. Therefore we think that portal vein air is a relative indication for operation. If a baby with portal vein air has any sign of instability, this suggests that early laparotomy should be performed.

With regard to Dr. Sheldon's comment, IgA is the glue that protects the bowel mucosa. It is the mucosal barrier, immunoglobulin. Premature babies are all immunosuppressed, however, and have decreased levels of IgA. They also have an immature mucosa that can absorb macromolecules. In addition premature infants have depressed levels of IgM, the main immunoglobulin *versus* gram-negative organisms, and their phagocytes do not destroy bacteria very well. The tiny premature baby is a very compromised host, which probably plays a significant role in the development of NEC insult.

Dr. Tapper's question referred to NEC possibly being a result of a reperfusion injury. We have not employed allopurinol or superoxide dismutase in cases of NEC. Although we have studied this concept in the laboratory, there are no good clinical data to suggest that these substances should be administered to premature patients in an attempt to prevent NEC.

Sixteen-year Experience With Aortic Root Replacement

Results of 172 Operations

NICHOLAS T. KOUCHOUKOS, M.D., THOMAS H. WAREING, M.D., SUZAN F. MURPHY, R.N., and JOHANNA B. PERRILLO, R.N.

From the Division of Cardiothoracic Surgery, Department of Surgery, Washington University School of Medicine, St. Louis, Missouri

During a 16-year interval ending in October 1990, 168 patients underwent 172 aortic root replacements. Thirty patients (18%) had Marfan syndrome. Annuloaortic ectasia (81 patients) and aortic dissection (63 patients) were the principal indications for operation. Twenty-seven patients (16%) had previous operations on the ascending aorta or aortic valve. The hospital mortality rate was 5% and the duration of cardiopulmonary bypass was the only significant independent predictor of early death ($p = 0.017$). Major modifications in technique were made in 1981, when the inclusion/wrap technique employing a composite graft (used in the first 105 procedures) was abandoned in favor of an open technique (used in 51 procedures), and in 1988, when aortic allografts and pulmonary autografts were introduced for selected conditions (reoperations, dissection, endocarditis, isolated aortic valve disease) in 16 patients. The mean duration of follow-up was 81 months. Forty-six patients were followed for more than 10 years. The actuarial survival rate was 61% at 7 years and 48% at 12 years. No significant difference in survival rate was observed between the patients with annuloaortic ectasia and aortic dissection, or between the inclusion/wrap and open techniques. However the frequency of pseudoaneurysm formation at suture lines and the frequency of reoperations on the ascending aorta and aortic valve were less with the open technique. The actuarial freedom from thromboembolism for the 152 patients with prosthetic valves was 82% at 12 years. One early and one late death occurred among the 16 patients with allograft or autograft root replacement. Anticoagulant therapy was not used in these patients and no thromboembolic episodes occurred in the follow-up period (mean, 7 months). The satisfactory results observed with extended follow-up support the continued use of the composite graft technique as the preferred method of treatment for patients with annuloaortic ectasia, persistent aneurysms of the sinuses of Valsalva following previous operations, and for patients with ascending aortic dissection who require aortic valve replacement. The availability of aortic root allografts and the perfection of techniques for safe implantation of the autologous pulmonary root into the aortic position have broadened the indications for aortic root replacement.

In 1963 Bentall and DeBono[1] described a technique for replacement of the ascending aorta and aortic valve using an aortic graft valve (composite) prosthesis. The original technique has been modified and has been widely used for a variety of pathologic conditions involving the ascending aorta and aortic valve. The recent availability of commercially prepared and cryopreserved aortic and pulmonic allografts and the perfection of techniques for allograft replacement of the aortic root and for implantation of the autologous pulmonary root in the aortic position, followed by replacement of the pulmonary root with a pulmonary autograft, have broadened the indications for aortic root replacement. In this report we present our entire experience with aortic root replacement in 168 patients during a 16-year interval.

Methods

Between September 1974 and October 1990, 168 patients underwent 172 operations to replace the ascending aorta and aortic valve in continuity (aortic root replacement). The results of operation on the first 127 patients, including 86 patients who were operated on by us and our former colleagues at the University of Alabama at Birmingham Medical Center, have been reported previously.[2] The mean age of the patients was 47 years (range, 16 to 85 years) and 118 (70%) were male. Thirty patients (18%) had clinical stigmata of Marfan syndrome.[3] The

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Address reprint requests to Nicholas T. Kouchoukos, M.D., Department of Surgery, Jewish Hospital at Washington University Medical Center, 216 S. Kingshighway, St. Louis, MO 63110.

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abnormalities of the ascending aorta and aortic valve requiring aortic root replacement are shown in Table 1. Annuloaortic ectasia (dilatation of the sinuses of Valsalva with associated moderate or severe aortic valve incompetence, cephalad displacement of the coronary ostia, and varying degrees of dilatation of the more distal descending aorta)[4] was the most common indication for operation (84 patients). Sixty-four patients had acute or chronic ascending aortic dissection. Twenty-eight of fifty-four patients with aortic dissection undergoing primary operations also had annuloaortic ectasia. Three patients had pathologic changes in the aorta compatible with syphilitic aortitis and associated aortic valve incompetence. Twenty-seven patients (16%) had previous operations on the ascending aorta, aortic valve, or both, a mean of 73 months (range, 3 to 142 months) before reoperation. Ten of these patients had previous graft replacement of the ascending aorta for acute or chronic dissection and required reoperation because of progressive aortic valve incompetence or development of aneurysms of the sinuses of Valsalva. Ten patients had previous operations for aortic valve disease or annuloaortic ectasia and at reoperation required aortic root replacement. These patients had progressive ectasia that was not present or was not treated at the initial operation. Five patients had pseudoaneurysms, four resulting from disruption of the aortic or coronary arterial suture lines and one from a spontaneous tear in the Dacron™ tube portion of the composite graft. Two patients had tunnel aortic stenosis that was treated initially by aortic valve replacement in one patient and composite graft replacement in the other.

Among the patients with annuloaortic ectasia, progressive aortic valve incompetence was the most common indication for operation. Eleven patients with annuloaortic ectasia, but without evidence for moderate or severe aortic valve regurgitation, had elective aortic root replacement because the greatest diameter of the aortic sinuses or ascending aorta exceeded 5.5 to 6.0 cm. The four patients with isolated aortic valve stenosis or incompetence who had aortic root replacement were severely symptomatic. Preoperative functional status was assessed in 170 patients. Twenty-seven patients were in New York Heart Association (NYHA) class I, 45 were in class II, 51 in were class III, and 47 were in class IV. Seventeen of the patients in class IV required emergency operation for acute dissection. Significant coronary artery occlusive disease (more than 50% stenosis of at least one major artery) was present in 23 patients (13%). Five patients had severe mitral valve incompetence.

During the study interval, 30 additional patients had supracoronary graft replacement of the ascending aorta and separate aortic valve replacement. Three of these patients had annuloaortic ectasia, four had acute aortic dissection, and 23 had aneurysms of the ascending aorta that did not involve the sinuses of Valsalva. These patients are not included in the subsequent analyses.

TABLE 1. *Indications for Aortic Root Replacement*

Operative Procedure			No. of Patients
Primary operations			
Annuloaortic ectasia			81
DeBakey type I dissection			37
Acute	16		
Chronic	21		
DeBakey type II dissection			17
Acute	1		
Chronic	16		
Syphilitic aortitis			3
Aortic stenosis			2
Aortic incompetence			2
Endocarditis			1
Aortic disruption after aortic valve replacement			1
Poststenotic dilatation			1
Reoperations			
Aortic sinus aneurysms after supracoronary graft replacement of aorta and/or aortic valve replacement for:			20
Dissection		10	
Aortic valve disease		7	
Aortic stenosis	2		
Prosthetic endocarditis	2		
Poststenotic aortic dilatation	1		
Periprosthetic leak	1		
Failed aortic allograft	1		
Annuloaortic ectasia		3	
Pseudoaneurysm			5
Tunnel Aortic Stenosis			2
Total			172

Operative Considerations

The general method for insertion of a composite graft by an inclusion/wrap technique (performing the distal aortic anastomosis inside the intact aorta and wrapping the aortic wall snugly around the aortic graft) has been previously described.[5] This technique was used in 105 procedures. A major modification was made in 1981. This consists of preclotting the prosthesis with 25% albumin solution, which renders the Dacron graft impermeable to blood, and abandonment of the inclusion/wrap technique.[6] After excision of the aortic valve and completion of the suture lines between the aortic annulus and the sewing ring of the prosthetic valve, buttons of graft opposite the coronary ostia are excised with a cautery. The aortic wall immediately adjacent to the coronary ostia is sutured to these openings with continuous 4-0 polypropylene suture. If there is minimal cephalad displacement of the coronary ostia, or if there is dissection with friable or separated aortic tissue surrounding the coronary ostia, the coronary arteries are detached from the aorta with a button of aortic tissue and after mobilization are anastomosed directly to the graft. The latter modification was used with increasing frequency in the later years of the study interval. If there is concern about the integrity of the coronary anastomoses, segments of saphenous vein or synthetic graft (woven Dacron™ or polytetrafluoroethylene) can be interposed between the tube graft and the coronary ostia. Alternatively the interposition graft can be anastomosed end to side to the coronary artery more distally. In this situation the coronary ostium is oversewn and the coronary graft is anastomosed proximally to the tube graft or to the aorta distal to the graft. After completion of the coronary anastomoses, the tube graft is cut to the appropriate length and the aorta is completely transected. If aortic dissection is present, the separated layers of the aorta are approximated between an outer and inner strip of Teflon™ felt with interrupted 4-0 polypropylene mattress sutures.[6] This cuff is then sewn to the graft with a continuous 3-0 polypropylene suture.

If replacement of the arch is necessary, a separate preclotted woven Dacron™ graft is sewn to the aorta distal to the left subclavian artery and to the brachiocephalic vessels during a period of circulatory arrest.[6] After these suture lines are completed, the graft is clamped proximal to the innominate artery, cardiopulmonary bypass is reestablished, and the composite graft is sewn in place. The two grafts are then approximated with 3-0 polypropylene suture. After completion and careful examination of all suture lines, cardiopulmonary bypass is discontinued and protamine is administered. No attempt is made to wrap the aorta tightly around the graft or to cover the entire graft because this can result in excessive tension on the graft to coronary ostial anastomoses and may contribute to the development of pseudoaneurysms, particularly if blood accumulates in the space between the graft and aorta. This general method was used in 51 procedures and is currently our preferred technique when a composite graft is used.

Reattachment of the coronary arteries using 10-mm Dacron™ grafts and suture of only the Dacron™ tube graft to the aortic annulus, placing the prosthetic valve within the tube graft in a more cephalad position, a technique described by Cabrol,[7] was used in one patient during the third composite graft replacement of the aortic root. Creation of a perigraft space-right atrial fistula, also described by Cabrol,[8] was not performed in any patient in whom the inclusion/wrap technique was used.

Beginning in 1988 cryopreserved aortic allografts and pulmonary autografts were used for selected conditions in 16 patients. Allograft replacement of the aortic root was used in eight patients using the technique described by Ross.[9] Four of these patients had malfunction of a previously inserted aortic valve, two had persistent tunnel aortic stenosis following aortic valve replacement or composite graft replacement, and two had aortic dissection (one acute, one chronic). Aortic root replacement with a pulmonary autograft combined with replacement of the pulmonary root with a pulmonary allograft was performed in eight patients using the technique described by Randolph et al.[10] Two of these patients had annuloaortic ectasia, two had isolated aortic valve incompetence, two had isolated aortic valve stenosis, one had endocarditis of the aortic valve with annular erosion, and one had a periprosthetic leak following two previous aortic valve replacements with mechanical valves.

Concomitant procedures were performed in 46 (27%) of the 172 patients (Table 2). Twenty-two patients required coronary artery bypass grafting (one to four grafts) for coronary artery occlusive disease. The left internal mammary artery was used in two of these patients. One patient had endarterectomy of the left coronary artery ostium because of atherosclerotic obstruction. Eight patients had coronary artery bypass grafts placed to the right coronary artery because of dissection and friability of the aortic tissue surrounding the right coronary ostium and one patient had 10-mm Dacron™ grafts interposed between the coronary ostia and the tube graft (see above). Six patients had replacement of the aortic arch using total circulatory arrest and five patients had mitral valve replacement.

Hypothermic potassium-induced crystalloid or blood cardioplegia was used in all but 27 patients and remains our method of choice for myocardial protection. The mean duration of cardiopulmonary bypass was 145 minutes and that of aortic occlusion was 103 minutes. In 26 patients woven tube grafts were sutured to various prosthetic valves (Starr-Edwards Model 1260, Baxter Healthcare, Edwards CVS Division, Santa Ana, CA) aortic prostheses (four patients), porcine xenografts (4 patients), Bjork-Shiley tilting disc prostheses (Shiley, Inc., Irvine, CA) (17 patients), or St. Jude tilting disc prosthesis (St. Jude Medical, Inc. St. Paul, MN) (1 patient). Composite grafts manufactured by Shiley Laboratories or St. Jude Medical were used in the remaining 130 patients. All but the four patients with bioprosthetic valves and the 16 patients with allograft or autograft valves received long-term anticoagulation with warfarin.

TABLE 2. *Concomitant Procedures in the 172 Patients*

Procedure		No. of Patients
Coronary artery bypass grafting for		31
Coronary atherosclerosis	22	
Technical problems	9	
Replacement of aortic arch		6
Mitral valve replacement		5
Endarterectomy of left main coronary artery		1
Closure of patent ductus arteriosus		1
Closure of atrial septal defect		1
Repair partial anomalous pulmonary venous connection		1
Total		46 (27%)

Follow-up

Follow-up information was available for all hospital survivors and was obtained by examination or by correspondence with the patient and referring physician. The date of last inquiry was between October 1989 and November 1990. The mean duration of follow-up for all hospital survivors was 81 months (range, 1 to 185 months). It was 93 months for the patients receiving composite grafts, and 7 months for the patients receiving aortic allografts or pulmonary autografts. Sixty-six patients have been followed for more than 7 years and 46 patients for more than 10 years.

Definitions

Thromboembolic events were defined as documented episodes resulting in transient or permanent central nervous system, visceral or peripheral ischemia. Valve thrombosis was analyzed separately. Complications related to anticoagulant therapy were defined as bleeding episodes necessitating hospitalization or blood transfusions. Infective endocarditis was considered present when septicemia necessitating reoperation was present, prolonged antibiotic therapy was required, or if it was found at autopsy.

Statistical Methods

Survival and event-free rates were calculated using the Kaplan–Meier method in SAS Procedure Lifetest (SAS Institute, Cary, NC).[11] The endpoints used were death, reoperation, and the development of valve-related complications. Only the first occurrence of any specified complication was considered in the analyses. The survival and event-free rates were compared with the generalized Wilcoxon test.[12]

Dichotomous variables were analyzed by the chi square and Wilcoxon rank-sum tests and continuous variables by the Van der Waerden Scores option of the Procedure NPAR1WAY in SAS (SAS Institute). Variables associated with increased risk of hospital and late death were assessed by univariate and multivariate logistic regression analyses. For the hospital mortality rate, the variables tested were age at operation, gender, presence of the Marfan syndrome, presence of acute dissection, previous operation on the ascending aorta or aortic valve, need for emergent operation, technique of insertion of the composite graft, operation on the aortic arch, concomitant coronary artery bypass grafting or mitral valve replacement, use of hypothermic cardioplegia, duration of cardiopulmonary bypass and of aortic clamping, and postoperative ventricular arrhythmias. For late death the variables tested were age at operation, gender, preoperative NYHA functional class, presence of Marfan syndrome, presence of acute dissection, presence of aortic valve incompetence, previous operation on the aortic valve, ascending aorta or aortic arch, and concomitant coronary artery bypass grafting or mitral valve replacement.

Results

Early Results

Mortality rate. The hospital mortality rate for the 172 procedures was 5.2% (9 patients) (Table 3). There were two deaths among the 93 patients with primary or repeat operations for annuloaortic ectasia (2.2%). One patient with severe chronic obstructive pulmonary disease died of respiratory insufficiency and pulmonary hemorrhage (6 days after operation). The other patient, a 21-year-old man with Marfan syndrome, class IV symptoms, and severe mitral valve regurgitation that required mitral valve replacement, died of ventricular fibrillation associated with low cardiac output 36 hours after operation. One of forty-seven patients with chronic dissection (2.1%) died of myocardial failure during operation. Four deaths occurred among the 16 patients with acute dissection (25%), one of myocardial failure during operation, one of intraoperative hemorrhage, one of ventricular fibrillation on the 12th postoperative day, and one of hemoptysis on the 24th postoperative day. The two deaths in the remaining 16 patients occurred from myocardial failure, during operation in one patient and after operation in the other patient following the fourth operation on the aortic root. One death occurred among the 27 patients having reoperations (3.7%). By univariate and multivariate analyses, duration of cardiopulmonary bypass was the only statistically significant predictor of hospital mortality rate (p = 0.017).

Morbidity rate. Reoperation for hemorrhage was required in 15 patients (8.7%). The incidence was 13.3% among the 105 patients in whom the inclusion/wrap technique was used and 2% among the 51 patients in

TABLE 3. *Hospital Mortality Data*

Aortic Disease	No. of Patients	Hospital Deaths No.	Hospital Deaths %	CL*
Annuloaortic ectasia	93	2	2.2	0.6–3.8
Chronic dissection	47	1	2.1	0–4.3
Acute dissection	16	4	25.0	13.8–36.2
Other	16	2	12.5	3.9–21.1
Syphilitic aortitis				
Endocarditis				
Poststenotic dilatation				
Tunnel aortic stenosis				
Aortic valve disease				
Prosthetic valve malfunction				
Total	172	9	5.2	3.4–7.0

* CL, 70% confidence limits.

TABLE 4. *Reoperations on the Ascending Aorta or Aortic Valve*

Indication for Reoperation	Total (n = 172)	Inclusion/Wrap Technique (n = 105)	Open Technique (n = 51)	Allograft or Autograft (n = 16)
Postoperative hemorrhage	15 (8.7%)	14	1	0
Pseudoaneurysm	10 (5.8%)	9	1	0
Endocarditis	4 (2.3%)	3	1	0
Technical problems	2 (1.2%)	2	0	0
Thrombotic obstruction	2 (1.2%)	2	0	0
Valve failure	1 (0.6%)	1	0	0
Subvalvular obstruction	1 (0.6%)	1		0
Subvalvular aneurysm	1 (0.6%)	0	1	0
	36 (21%)	32 (30%)	4 (8%)	0

whom the open technique was used ($p = 0.024$). None of the 16 patients in whom aortic allografts or pulmonary autografts were used required reoperation for this complication (Table 4). One patient in whom the inclusion/wrap technique was used required reoperation early after operation for prosthetic valve incompetence that resulted from compression of the tube graft immediately above a Bjork-Shiley prosthesis. Thrombus accumulating between the graft and the aortic wall prevented closure of the disc. The thrombus was evacuated and a Dacron™ patch was sutured to the aortic wall to relieve the compression. The patient recovered with no complications.

Thirty-one patients (18%) required inotropic support for more than 6 hours after operation. Intra-aortic balloon pumping was required for 5 of the 168 operative survivors (3%). One patient had insertion of temporary left and right ventricular assist devices following a fourth operation on the aortic root. Ventricular arrhythmias necessitating cardioversion or drug therapy occurred in 33 patients (19%). Permanent pacemakers were inserted in five patients. Four patients required tracheostomy and prolonged ventilatory support. New focal neurologic deficits developed in eight patients after operation. Three of the deficits were permanent.

Postoperative aortograms were obtained in 25 patients before hospital discharge. No abnormalities of the proximal or distal aortic anastomoses or the graft-coronary ostial anastomoses were noted in 19 patients. This included 8 of the 47 hospital survivors in whom the open technique was used. Pseudoaneurysms were observed at the left coronary ostial anastomosis in four patients and at the distal aortic anastomosis in two patients. The inclusion/wrap technique was used in all six patients. Repair of the pseudoaneurysm at the aortic suture line was performed in one patient before hospital discharge. None of the other five patients underwent early reoperation. Stenosis of the left coronary ostium was noted in one patient. This was corrected with a saphenous vein bypass graft to the left anterior descending artery 12 weeks after operation. The inclusion/wrap technique was used in this patient. Seven of the 25 patients had DeBakey type I dissections, and persistent dissection was present in the aortic arch and descending aorta.

Late Results

Mortality rate. There have been 58 late deaths (Table 5). Thirteen of these deaths (22%) were related to the graft valve prosthesis. Four patients died after operation to repair a pseudoaneurysm, four of prosthetic endocarditis (two after reoperation), and five of stroke (one of a cerebral embolus and four from cerebral hemorrhage). Eight patients died suddenly, five died of documented arrhythmias, and four died of ischemic heart disease. Three patients died of aneurysmal disease of the residual aorta. The cause of death was unknown in 17 patients.

Actuarial survival of the 168 patients is shown in Figure 1. The survival rate was 61% at 7 years and 48% at 12 years. Survival curves of the 81 patients with annuloaortic ectasia and the 63 patients with aortic dissection are shown

TABLE 5. *Causes of Late Death*

Prosthesis related		13
Pseudoaneurysm	4*	
Endocarditis	4†	
Stroke	5	
Sudden		8
Arrhythmias		5
Ischemic heart disease		4
Renal failure		3
Trauma		2
Cancer		2
After replacement of arch aneurysm‡		2
Rupture of other aneurysm		1
Respiratory failure		1
Unknown		17
Total		58

* Three died at reoperation.
† Two died at reoperation.
‡ One died at reoperation.

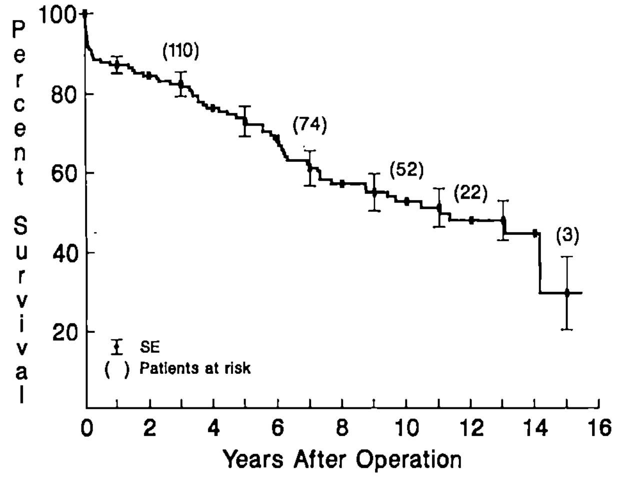

FIG. 1. Actuarial survival rates of the 168 patients. In this and subsequent figures, the vertical bars enclose the standard error (SE). The numbers in parentheses indicate the number of patients traced at that time.

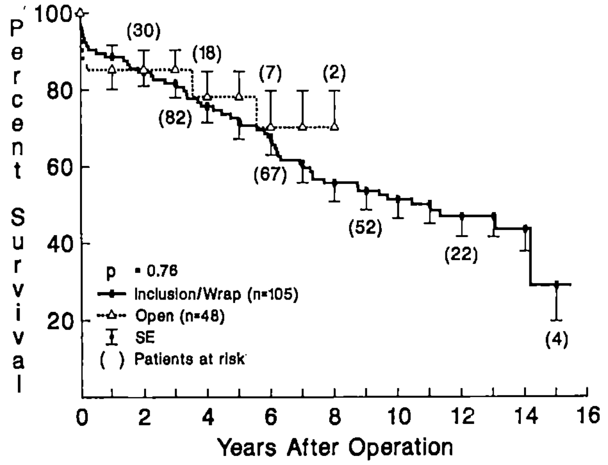

FIG. 3. Actuarial survival rates according to type of procedure (inclusion/wrap or open). The three patients who underwent four repeat aortic root replacements are included only once in the analysis.

in Figure 2. The 7-and 12-year survival rates were higher for the patients with annuloaortic ectasia, 67% and 54%, respectively, than for the patients with aortic dissection, but the differences were not statistically significant (p = 0.12). Survival according to operative technique for the 153 patients undergoing prosthetic replacement of the aortic root is shown in Figure 3. There was no significant difference between the two operative techniques. The 1-year actuarial survival rate for the 16 patients having aortic root replacement with aortic allografts or pulmonary autografts was 86%, which is similar to that for the inclusion/wrap and open techniques. The survival rate of the 30 patients with Marfan syndrome was lower than that for the remaining 138 patients at 12 years (44% *versus* 49%, respectively), but the difference was not significant (p = 0.22) (Fig. 4). By multivariate analysis, increased age (p = 0.001), presence of Marfan syndrome (p = 0.002), and increasing NYHA functional class (p = 0.007) were significant independent predictors of late death.

Reoperations on the ascending aorta. Aortograms were performed in 24 patients from 1 to 155 months after hospital discharge. Pseudoaneurysms of the aortic or coronary ostial suture lines were identified in nine patients and the inclusion/wrap technique was used in all of them (Table 4). Reoperation was performed in the nine patients 1 to 145 months (mean, 47 months) after operation and five survived. Three of the nine patients had Marfan syndrome. One patient in whom the open technique was used developed a pseudoaneurysm that resulted from two spontaneous tears in the Dacron® tube graft. He under-

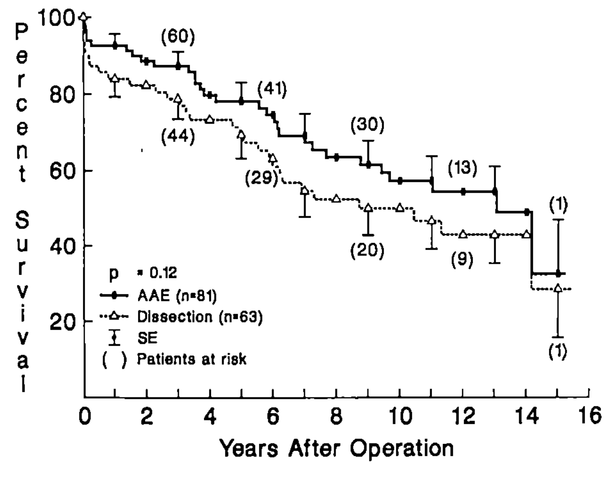

FIG. 2. Actuarial survival rates of the 81 patients with annuloaortic ectasia and the 63 patients with aortic dissection.

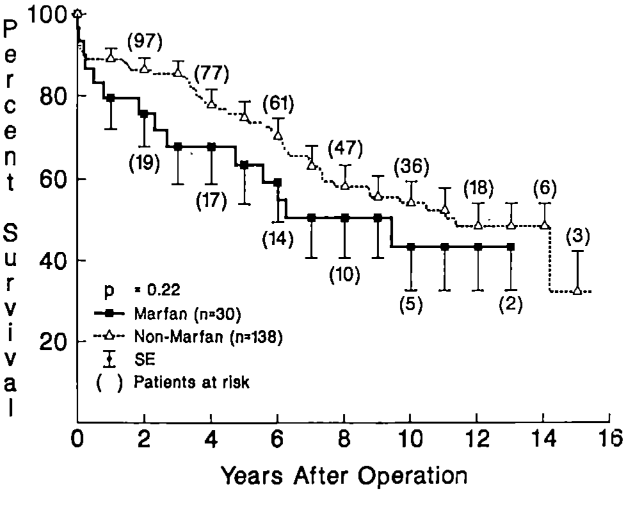

FIG. 4. Actuarial survival rates for the 30 patients with the Marfan syndrome and for the other 138 patients. The three patients who underwent four repeat aortic root replacements are included only once in the analysis.

went successful replacement of the composite graft 51 months after the initial operation. Four patients required reoperation for prosthetic endocarditis between 1 and 19 months after operation. The composite graft was replaced with another composite graft in three of these patients and with separate aortic valve and ascending aortic graft prostheses in the fourth patient. Two of the four patients survived reoperation. Two patients had thrombotic obstruction of a Bjork-Shiley valve 23 and 56 months after operation. Warfarin therapy had been discontinued in both patients. Both had successful replacement of the graft valve prosthesis. One patient developed stenosis of a porcine aortic bioprosthesis and had a valved conduit inserted from the left ventricular apex to the descending thoracic aorta, one patient developed progressive subvalvular (tunnel) aortic stenosis and had replacement of the composite graft with an aortic allograft and enlargement of the aortic outflow tract, and one patient developed an aneurysm of the left ventricular outflow tract that was treated by patch closure and replacement of the composite graft.

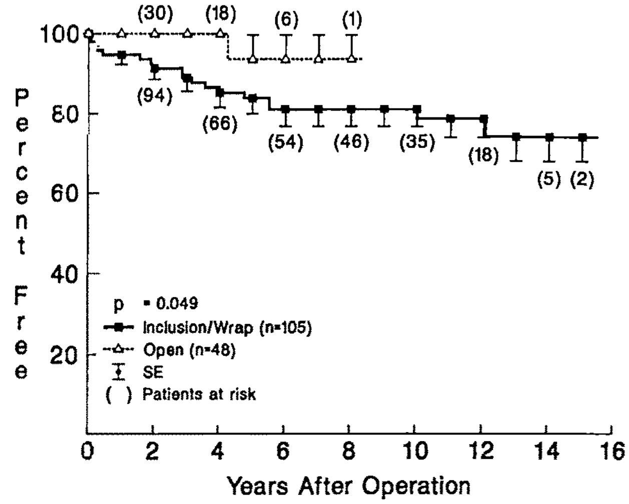

FIG. 6. Actuarial freedom from reoperation on the ascending aorta or aortic valve (exclusive of early reoperations for hemorrhage) according to operative technique for the patients receiving prosthetic grafts.

The probability of freedom from reoperation on the ascending aorta or aortic valve for any cause for the two operative techniques in the patients receiving prosthetic grafts is shown in Figure 5. At 5 years 92% of the patients in whom the open technique was used and 71% of the patients in whom the inclusion/wrap technique was used did not require reoperation (p = 0.003). When early reoperations for hemorrhage were excluded, the difference at 5 years remained significant (94% *versus* 84%) (p = 0.0498) (Fig. 6). No patient having aortic root replacement with an allograft or autograft has required reoperation. The probability of freedom from reoperation for pseudoaneurysms of the aortic or coronary ostial suture lines for the two operative techniques using prosthetic grafts is shown in Figure 7. No patient in whom the open technique was used has required reoperation for this complication. Ninety per cent of the patients in whom the inclusion/wrap technique was used did not require reoperation at 7 and 12 years. One patient in this group required reoperation for this complication 145 months after operation. The difference between the two groups was not statistically significant (p = 0.13).

Operations on the remaining aorta. Ten patients have required subsequent operations for aneurysmal disease or dissection of the remaining thoracic or the abdominal aorta 1 to 157 months after operation. At 7 years, the

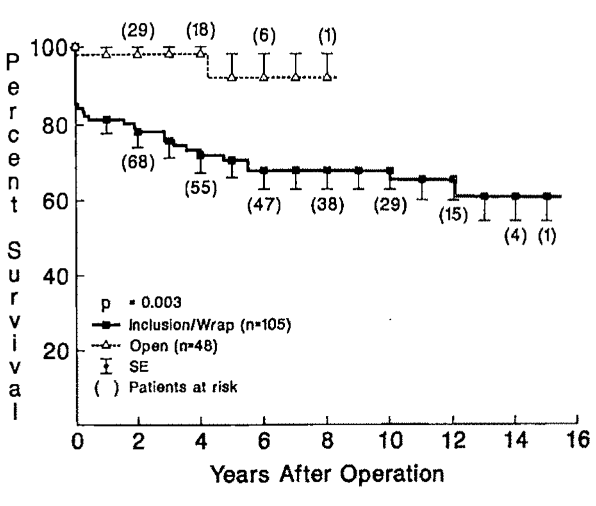

FIG. 5. Actuarial freedom from reoperation on the ascending aorta or aortic arch for any cause according to operative technique for the patients receiving prosthetic grafts.

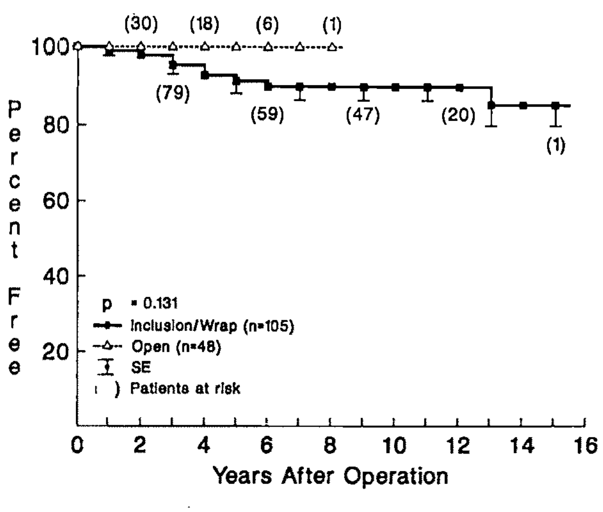

FIG. 7. Actuarial freedom from reoperation for pseudoaneurysms of the aortic or coronary ostial suture lines according to operative technique for the patients receiving prosthetic grafts.

actuarial probability of freedom from operation was 85% for the patients with Marfan syndrome and 96% for the remaining patients. At 12 years these rates were 55% and 96%, respectively ($p = 0.002$) (Fig. 8).

Thromboembolism. Thromboembolic events occurred in 16 of the 152 patients receiving prosthetic grafts and one was fatal. At 12 years the actuarial probability of remaining free from a thromboembolic event was 82% (Fig. 9). Except for the four patients with porcine xenografts and the two patients who developed thrombotic obstruction of the aortic prosthesis after cessation of oral anticoagulants, all patients were receiving warfarin therapy and were having prothrombin times obtained at regular intervals. Warfarin was not used in the 16 patients who received allografts or autografts and no thromboembolic episodes have occurred in the follow-up interval. Eighteen patients described transient visual disturbances, including blurring of vision, diplopia, and focal blind spots that usually resolved within minutes. Because they were transient and totally reversible, they were not considered as thromboembolic events. A similar incidence of this complication in patients having composite graft replacement of the aortic root has been reported by Peigh et al.[13]

Anticoagulant-related complications. Thirteen patients had complications related to anticoagulant therapy and four were fatal. The actuarial probability of remaining free of these complications was 91% at 12 years (Fig. 10).

Prosthetic endocarditis. Seven patients developed prosthetic endocarditis. Four patients underwent reoperation (two survivors) and three were treated medically (one survivor).

Event-free survival rate. The actuarial probability of being alive and free of a prosthesis-related complication for the patients receiving prosthetic grafts was 55% at 7 years and 38% at 12 years (Fig. 11).

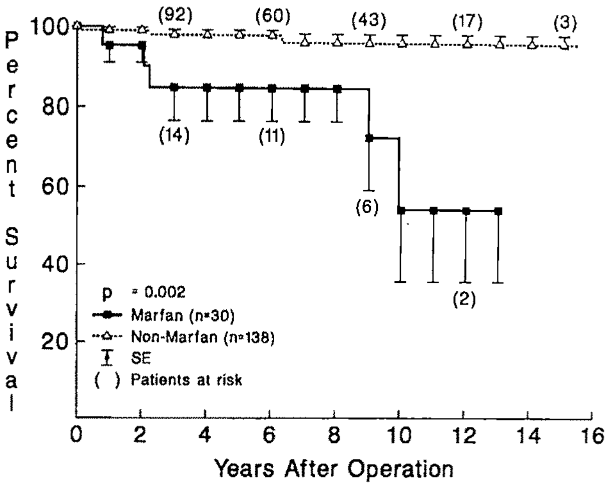

FIG. 8. Actuarial freedom from reoperation on the remaining aorta for the 30 patients with Marfan syndrome and for the other 138 patients.

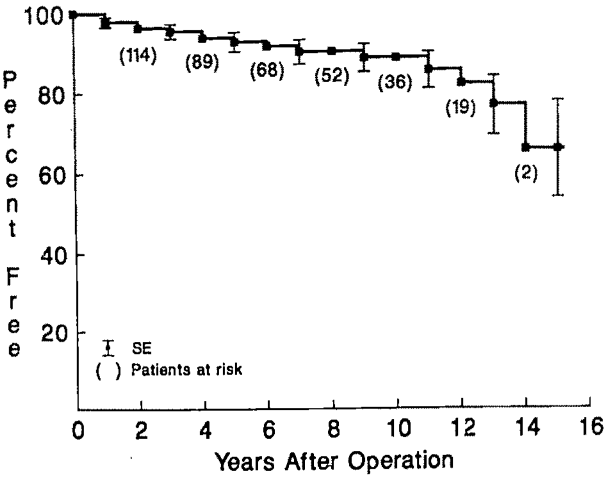

FIG. 9. Actuarial freedom from thromboembolism for the 153 patients receiving prosthetic grafts.

Symptomatic status of hospital survivors. At the time of last follow-up of the 159 hospital survivors, 58 were dead, 75 (74%) were in class I, 16 (16%) were in class II, 9 were in class III, and 1 was in class IV.

Discussion

The results of this study confirm our previous observation and that of others that elective replacement of the aortic root in patients with annuloaortic ectasia or chronic aortic dissection can be accomplished with low operative risk.[2,14-23] In the present series, duration of cardiopulmonary bypass was the only significant independent predictor of hospital death. Reoperation on the aortic root was not associated with an increased hospital mortality rate.

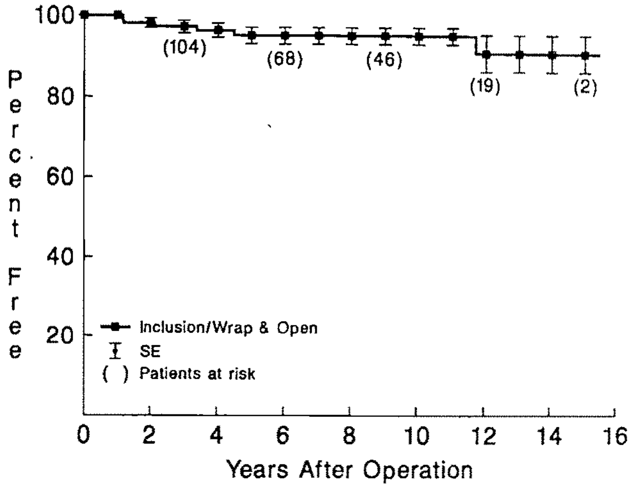

FIG. 10. Actuarial freedom from complications related to anticoagulant therapy for the 149 patients who received warfarin therapy.

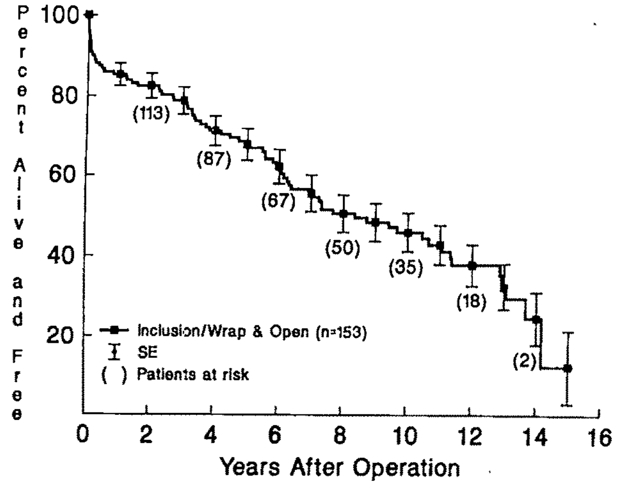

FIG. 11. Actuarial survival free of graft related complications for the 153 patients receiving prosthetic grafts.

Reoperation

Major modifications in operative technique were made in 1981, when the inclusion/wrap technique was abandoned in favor of an open technique, and in 1988 when aortic allografts and pulmonary autografts were first used. The frequency of reoperation for postoperative hemorrhage was significantly reduced with the open technique of composite graft replacement compared to the inclusion/wrap technique (13% *versus* 2%). No reoperations for hemorrhage were required among the patients who received allografts or autografts. While increasing operative experience may be a major factor responsible for this reduction in serious postoperative hemorrhage, other factors (*i.e.*, preclotting of the grafts with albumin, improved pump oxygenator systems, accurate heparin/protamine titration) have undoubtedly contributed to the reduction as well. Thus the need for wrapping the aorta tightly around the graft to minimize bleeding, as originally advocated by Bentall and De Bono, has been obviated.[24]

In our experience development of pseudoaneurysms at the aortic and coronary ostial suture lines has been observed only with the inclusion/wrap technique. Crawford et al.[25] have had a similar experience. Nine of the one hundred five patients in our series in whom this technique was used required reoperation for such pseudoaneurysms and five (56%) survived. In other series the incidence of pseudoaneurysms with this technique has ranged from 7% to 25%.[15,26-28] Because aortography is not performed on all patients after root replacement, the true frequency of pseudoaneurysm formation is unknown and may be higher. A long interval between the development of symptoms and need for reoperation is not unusual. In our series the mean time to reoperation was 47 months. One patient required reoperation for this complication 145 months after the initial operation. The four deaths after operative repair of the pseudoaneurysm occurred in patients in whom the aneurysms were large and were adherent to the sternum, and in whom urgent or emergent operation was required. In follow-up that extends to 8 years, none of the 47 hospital survivors in whom the open technique was used has required reoperation for pseudoaneurysm of the aortic or coronary ostial suture lines (Fig. 7), no pseudoaneurysms were observed in the 12 patients who had postoperative aortograms, and no patient has roentgenographic findings that indicate pseudoaneurysm. Absence or a low incidence of such pseudoaneurysms has been observed in other series in which the inclusion/wrap technique was not used and in which postoperative aortography was used to assess the frequency of this complication in most hospital survivors.[14,19,22] Because of the structural abnormalities in the aortas of patients with annuloaortic ectasia and dissection, particularly those with Marfan syndrome, pseudoaneurysms may develop with other techniques besides the inclusion/wrap technique in the postoperative period.[18,29] Other problems, such as graft failure, which was noted in one of our patients in whom the open technique was used, can also result in pseudoaneurysms. For these reasons, periodic evaluation of all patients following aortic root replacement is necessary. When pseudoaneurysms are detected, they should be repaired, preferably before they reach large size, become adherent to the undersurface of the sternum, or erode into cardiac chambers and require emergent operation. In such situations operation is associated with substantial risk.[25,30]

The modification described by Cabrol of interposition of 8- to 10-mm Dacron™ grafts between the tube graft and the coronary ostia was used in only one patient in our series during a third reoperation on the aortic root. We avoid placement of prosthetic grafts in the coronary circulation whenever possible. However this modification is of particular value when, because of significant aneurysmal enlargement of the sinuses of Valsalva or marked fibrosis, the coronary ostia cannot be safely attached to the tube graft. We have not found it necessary to create a fistula between the perigraft space and the right atrium for control of hemorrhage, although we recognize that this maneuver sometimes can be lifesaving.[25,31,32]

A high incidence of subsequent operations on the remaining aorta was observed among the patients with Marfan syndrome (Fig. 8). A similarly high incidence of operation on the residual aorta in patients with Marfan syndrome before or after operations on the ascending aorta or aortic valve was reported by Crawford.[33] In a larger series of patients having surgical treatment of aneurysms and dissections of the ascending aorta and transverse arch, Crawford et al.[25] identified disease of the ascending aorta and arch as frequently a part of a more diffuse process and that extensive graft replacement, not infrequently involving the entire aorta, is necessary for successful management. These findings also emphasize the need for periodic evaluation of the aorta for the lifetime of the patients.

Survival Rate

The late survival rate for the 168 patients in our series is similar to that reported in other series using composite or separate graft valve techniques.[8,15,19,21,25,29] The proportions of patients with annuloaortic ectasia, Marfan syndrome, aortic dissection, and other characteristics differ in these series, so precise comparison with our patients is difficult. We found no significant difference in survival rates between the patients with annuloaortic ectasia and those with aortic dissection (Fig. 2). The lower rate of survival of the patients with aortic dissection was related primarily to a higher operative mortality rate among the patients undergoing operation for acute dissection (Table 3). Similarly no significant difference in survival was noted between the patients with and without Marfan syndrome or between those patients in whom the inclusion/wrap and open techniques were used.

Performance of the Valve and Aortic Substitutes

The Bjork-Shiley tilting disc prosthesis was used in 146 of the 156 patients in whom a composite graft was inserted. Long-term studies evaluating the performance of the Bjork-Shiley prosthesis in patients having isolated aortic valve replacement have shown survival and event-free survival rates at 10 to 12 years, and rates of freedom from thromboembolism, major complications related to anticoagulant therapy, valve thrombosis, and prosthetic endocarditis that are similar to those observed in our study with composite grafts.[34,35] Thus the incidence of these complications does not appear to be higher with composite graft replacement than for isolated aortic valve replacement. Transient visual disturbances and neurologic abnormalities have been observed with greater frequency following composite graft replacement of the ascending aorta and aortic valve than after separate ascending aortic and aortic valve replacement.[13] They may be related to stasis of blood in the sinus portion of the composite graft and appear to decrease in frequency with time. We and others have found that antiplatelet therapy using dipyridamole (50 to 75 mg three times a day) reduces the frequency of these events.[2,13]

Although the follow-up for the 14 survivors of allograft or autograft replacement of the aortic root does not exceed 22 months, the performance of these grafts to date has been excellent. Serial echocardiographic studies have shown satisfactory function of the grafts, with minimal gradients across the valves, and no or only mild valve insufficiency that has not been progressive.[36] There have been no graft-related deaths or morbidity and anticoagulant or antiplatelet therapy has not been necessary. Because the pulmonary root is autologous tissue, there is potential for growth, and this would be a particular advantage for children who require aortic root replacement. The pulmonary root is probably not a satisfactory aortic root substitute for patients with Marfan syndrome because the connective tissue abnormalities that occur with this disorder are present in the pulmonary artery. A low frequency of valve failure and of reoperation to replace the pulmonary allografts that are used to replace the pulmonary root has been shown by Ross (81% free of valve failure or reoperation at 19 years).[37] Reoperation will be necessary for some patients who undergo aortic root replacement with aortic allografts.[38] However an interval free of thromboembolism and the need for anticoagulant therapy may be a particular advantage for younger patients or patients in whom anticoagulant therapy is inadvisable or contraindicated.

Our extended experience with composite graft replacement of the aortic root supports its continued use as the method of choice for patients with annuloaortic ectasia, persistent aneurysm of the sinuses of Valsalva after previous operations on the ascending aorta or aortic valve, and for patients with ascending aortic dissection who require aortic valve replacement, particularly when there is associated annuloaortic ectasia. In our experience use of the open technique has been associated with a lower incidence of pseudoaneurysms at the suture lines and of reoperations on the ascending aorta and aortic valve than with the inclusion/wrap technique and remains our preferred method when a composite graft is used. The use of aortic allografts and pulmonary autografts for selected conditions has broadened the indications for aortic root replacement, and in the short term is associated with a lower incidence of graft-related complications.

Acknowledgments

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DISCUSSION

DR. FRANK SPENCER (New York, New York): This one slide, prepared by Dr. Galloway at NYU, is the database from which my comments are made. Over a period of 12 years, about 200 aortic arch aneurysms have been treated surgically. Conduits were used in about 45.

Several points are of particular interest.

First the conduit procedure is clearly a very safe operation with a low operative mortality. Dr. Kouchoukos' data includes all operations, but if the small number with acute dissections are excluded, the mortality rate for elective procedures is only about 2%. Our mortality rate is somewhat higher, but has steadily decreased. There have been no deaths with the conduit procedure at our institution over the past 3 years.

Over the past few years, however, our frequency of the use of conduits has significantly decreased. A conduit is used primarily for dilatation of the aortic annulus with displacement of the coronary ostia cephalad for over 1 cm. This is found usually in the Marfan's syndrome or annuloectasia. It has not been used routinely with dissecting aneurysms unless the coronary ostia are significantly displaced. If the coronary ostia are not significantly displaced, the aortic valve is replaced, after which the Dacron graft is anastomosed to the aorta just above the coronary ostia, leaving the aortic sinuses intact.

With acute dissections the displaced aortic valve is usually reconstructed by resuspending it, using a similar technique to that for insertion of an aortic valve homograft. One of my questions for Dr. Kouchoukos is, what is the reason for their frequent use with chronic dissections? It probably does not make much difference because results are excellent with either technique.

A particularly interesting question is the benefits or hazards from wrapping the aortic Dacron graft with the aneurysmal wall, the original technique described by Bentnall. Our technique is virtually completely opposite to Dr. Kouchoukos', for the aortic graft is wrapped in virtually every patient. We have been quite pleased with the results. Dr. Kouchoukos' data indicate a significant decrease in frequency of false aneurysms when the wrap technique was abandoned, but, as with many changes in technique, an improvement in results cannot always be attributed to the change unless the study is randomized.

Several other changes have occurred that are probably the most significant. Smaller needles are used for the aortic anastomoses, using 4–0 for the aortic anastomoses, virtually never using 3–0. The bleeding is significantly less. 5–0 prolene is used for the coronary anastomoses.

A separate major advance, which I personally consider a very significant one, is the performance of the distal aortic anastomosis during temporary circulatory arrest with hypothermia. On bypass the patient is cooled for 15 to 20 minutes to a tympanic membrane temperature below 20 C. No clamps are applied to the distal aorta. Under circulatory arrest with an open technique, an unhurried distal anastomosis can be done at the level of the innominate or slightly beyond. This permits placing the suture line in healthy tissue and avoids the potential injury from application of an aortic clamp. Depending on the level of the disease, this open technique is used in about 60% of patients and has been strikingly free from complications.

With these techniques combined with woven grafts baked in albumin, there is much less bleeding from the Dacron grafts than previously. The frequency of false aneurysms with the distal suture line is about 2%. To date we have not recognized a false aneurysm at the coronary anastomosis.

Like Dr. Kouchoukos, we have not found it necessary to use a Dacron tube to connect to the coronary ostia. The Cabrol technique of connecting the sac of the aneurysm to the right atrium as a side-to-side anastomosis is used perhaps once a year.

Over the past 1 to 2 years, coronary sinus cardioplegia has virtually displaced antegrade cardioplegia. This greatly simplifies the operation, as intermittently stopping the procedure for perfusion of the coronary arteries is not necessary. The heart temperature remains below 15 C. Whether this change in preservation influences the late mortality rate is a significant point for future considerations. Dr. Kouchoukos' data show a high percentage of late cardiac deaths, about 6% a year, with about 60% of patients alive 7 years after operation. Whether the late cardiac mortality is due to irreversible preoperative cardiac injury or to subclinical injury from inadequate preservation is unknown.

The frequency of strokes is a particularly important consideration, as this is the most serious morbidity. In our series of 200 patients, four strokes have occurred. There are eight in the series reported by Dr. Kouchoukos. Hence my second question is whether he has any suggestions regarding decreasing their frequency. Not putting a clamp on the distal aorta may be helpful in this regard.

Like Dr. Kouchoukos we have begun to use pulmonary homografts and autografts in selected patients and have currently used these in about 15. The big attraction of pulmonary autografts, as originally developed by Ross, is the striking freedom from emboli.

DR. JOHN A. WALDHAUSEN (Hershey, Pennsylvania): Dr. Kouchoukos and his associates are to be congratulated on a superb series of patients with aortic root replacement. Their results are outstanding. In those with annuloaortic ectasia and chronic dissection, the mortality rate was 2.1%, a rate thought by most to be quite good in simple aortic valve replacement. The follow-up has been extensive and the results were fully analyzed.

The authors have clearly demonstrated that the original inclusion/wrap technique is inferior to the open technique and results in a significantly higher incidence of reoperation. We too have abandoned the inclusion/wrap technique for the open technique.

I was particularly interested in their use of the autograft replacement of the aortic root and the insertion of an allograft in the pulmonary trunk. Their immediate and relatively early results were excellent. I have some concerns with this procedure, however. It ultimately could well lead to a need to replace, at some later date, both aortic and pulmonary roots. Prosthesis-related reoperations in the authors' series did carry a higher risk for death.

Perhaps one can justify the use of an autograft in younger patients where anticoagulants are a greater inconvenience or even a hazard with a physically active life. This is particularly true for women who wish to become pregnant. In the very young, the potential for growth of the autograft is also a highly desirable advantage.

In most patients, however, I personally would prefer a St. Jude valve prosthesis with a Dacron conduit. The latter would, at least from what we currently know, have the least risk for reoperation.

Dr. Kouchoukos, how old were the patients that received the aortic allograft, the aortic autograft, and pulmonary allograft?

DR. HASSAN NAJAFI (Chicago, Illinois): It is indeed very gratifying that there has been significant decline in the risk associated with thoracic aortic surgery.

About a year ago, I presented before the Chicago Surgical Society my personal experience with 47 thoracic aortic procedures, in 45 patients operated on in the preceding 4 years. Two women in their 70s, with severe obstructive and chronic lung disease and renal insufficiency, did not survive, for a mortality rate of 4.2%.

While preparing this report, I noted that there had been no deaths in 19 consecutive patients having had conduit insertion, including several who had reoperations or had surgery extended into the transverse aortic arch.

I come to the podium primarily to congratulate Dr. Kouchoukos and his colleagues for their continued tradition of excellence in the work they do, and secondly to share with you an excitement in performing this operation in the last five patients without transfusion or the use of blood products. This is in spite of avoiding preclotting, which has been my practice in the past two and a half decades. For those of us who began doing this type of surgery late in the 1960s and early 1970s, and experienced the grief, trials, and tribulations that were associated with it, the progress in this particular surgery has been most rewarding.

Because most patients with chronic aneurysm of the ascending aorta, associated with aortic valve malfunction, are operated on electively, and because the majority are young and otherwise healthy, one or two units of blood could be drawn within 2 weeks before surgery. If necessary the use of this blood would reduce the need for homologous blood and blood products. Autotransfusion is not new to cardiac surgeons, but at least in my own personal experience, it has been a novelty in patients undergoing insertion of conduit in the last year.

DR. NICHOLAS T. KOUCHOUKOS (Closing discussion): Dr. Spencer asked why we remain enthusiastic about the use of the composite graft in chronic aortic dissection. Over half of the patients we operated on for acute or chronic dissection had annuloaortic ectasia, and we believe this is a firm indication for use of a composite graft. In other patients with acute dissection or chronic dissection who have substantial separation of the layers of the aorta in the region of the aortic sinuses, or who have dissection that involves the origin of the coronary arteries, we have had greater success with composite replacement and use of interposition grafts, when necessary, to reconstruct the coronary arteries than with the more conventional technique of approximating the disrupted layers of aorta. In our experience the incidence of serious bleeding problems has been reduced with the composite graft technique.

We agree with Dr. Spencer that use of hypothermic circulatory arrest and an open technique for performance of the distal graft-to-aorta anastomosis has been a major factor in reducing the early and late mortality and morbidity rates after surgical treatment of acute ascending aortic dissection.

Because the inclusion/wrap and the open techniques were used sequentially rather than concurrently, we cannot state with certainty that the better results observed with the open technique are due to the technique itself or to increased experience. Modifications in technique have been introduced in the last 10 years, however, that have improved the results. One of the most important, in my view, has been the preclotting of the Dacron grafts, currently with albumin and autoclaving, which has virtually eliminated bleeding through the grafts. This has made it easier to identify discrete bleeding points at the suture lines. Bentall initially used an inclusion technique and wrapped the aortic wall snugly around the graft to reduce bleeding, which had previously been a major cause of mortality and morbidity. With current techniques, we believe wrapping is no longer necessary, and can be disadvantageous because of the potential for development of pseudoaneurysms. If the inclusion/wrap technique is used, the aorta should not be wrapped around the graft until protamine has been administered and all suture lines have been carefully examined.

Dr. Waldhausen asked about the indications for use of the pulmonary autograft. We share his concern about the long-term results with this conduit. We believe it is an important alternative to root replacement, however, with a composite graft containing a mechanical valve in a child or adolescent, where there is potential for growth of the autograft, in young adults, especially women in the child-bearing age, in whom the use of anticoagulant therapy is undesirable, and in patients in whom the use of anticoagulants is contraindicated. Until more information is available on the long-term function of these grafts and of the pulmonary allografts used to replace the pulmonary root, we are using this procedure very selectively.

Dr. Najafi pointed out the important advances in autotransfusion technology that have reduced the requirements for homologous blood for aortic surgery. We have patients who are to undergo elective operations donate two or three units of blood preoperatively. It is then possible to perform aortic root replacement in these patients without the need for homologous blood products.

Second-look Surgery for Colorectal Cancer

The Second Time Around

EDWARD W. MARTIN, JR., M.D., and LARRY C. CAREY, M.D.

From the Department of Surgery, University of South Florida College of Medicine, Tampa, Florida

Eighty-six colorectal cancer patients who entered the Radioimmunoguided Surgery™ (RIGS®) protocol study were evaluated for 2-, 3-, 4-, and 5-year survival following second-look surgical procedures. Strict preoperative evaluation criteria eliminated patients with extra-abdominal tumor involvement. A saturated potassium iodide preparation was given before the intravenous administration of the B72.3 monoclonal antibody (1 mg) radiolabeled with 2 mCi of iodine-125 by the IODOGEN method. Precordial monitoring of the biologic clearance by the handheld, gamma-detecting probe (Neoprobe® 1000 instrument) was conducted at weekly intervals until the average count was less than 20 counts in 2 seconds. Once the drug cleared from the blood, surgery was performed. The mean time interval between injection and operation was 24 days, with a range of 21 to 28 days and a median of 23 days. At surgery the abdomen was explored through the traditional methods of palpation and inspection, and the surgeon committed to a planned procedure. The abdomen was then re-explored with the handheld, gamma-detecting probe and the surgeon stated another intraoperative assessment. After using both traditional and RIGS detection methods, the surgeon stated whether his or her surgical plans changed because of the additional intraoperative information provided by the RIGS system. Fifty-three patients (62%) were deemed resectable by the traditional methods of palpation and inspection, but only 40 (47%) were specified as resectable by RIGS exploration. Two-, three-, four-, and five-year survival data could be gathered for each of the three groups: RIGS resectable (n = 40), traditional nonresectable (n = 33), and RIGS nonresectable (n = 13). At 2 years 95% of the resectable, 36% of the traditional nonresectable, and 53% of RIGS nonresectable patients survived. At 3 years 83%, 7%, and 30% of these patients survived, respectively. For the resectable patients, 74% survived at 4 years and 60% at 5 years, with no survivors from either nonresectable group. Use of the RIGS system increased accurate selection of resectable patients undergoing second-look surgery for recurrent colorectal cancer.

THE CONCEPT OF second-look surgery was introduced 40 years ago by Wangensteen and colleagues.[1] Much has happened in those 40 years to improve on the initial results of 50% negative explorations and the 6.2% 5-year patient survival rate.[2] Radioimmunoassays, better anesthetic agents, improved surgical retractors, new surgical instruments, better preoperative imaging capabilities, and flexible endoscopes all have contributed to better survival rates with corresponding lower morbidity and mortality rates.

Increasing evidence in the literature supports the premise that major surgical ablation of recurrent tumor has led to improved survival rates and can be achieved with improved safety (less than 5% mortality rate).[3] With the improving imaging techniques, such as computed tomography (CT) scans, magnetic resonance imaging scans, and three-dimensional images, better selection of surgical candidates for second-look procedures has evolved, leading to improved survival rates. A better understanding of tumor markers (*e.g.*, carcinoembryonic antigen [CEA]) and improved antibody development (monoclonal antibodies) also have led to better earlier detection of recurrent tumor, often before the patient becomes symptomatic.[4,5]

Along with this better preoperative assessment of recurrent colorectal tumor, the improved intraoperative tools the surgeon now possesses are equally important. The intraoperative ultrasound device, the handheld gamma detector (Neoprobe 1000 instrument, Neoprobe Corp., Columbus, OH) and the Cavitron Ultrasonic Surgical Aspirator (CUSA) dissector (Cavitron Valley Lab, Boulder, CO) have all added information to the surgeon's traditional exploration by touch and sight, contributing to safe and more complete reoperations. Additional information obtained with these devices during the surgical procedure can lead to the removal of more tissue (occult tumor) not found by the traditional methods of palpation and inspection.[6]

This more complete intraoperative information allows the surgeon to decide with more assurance which patients should undergo an immediate major surgical procedure

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Address reprint requests to Larry C. Carey, Department of Surgery, University of South Florida College of Medicine, 4 Columbia Dr., Harbourside Medical Tower 430, Tampa, FL 33606.

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and which patients are not appropriate candidates for a curative procedure.

It has been our approach that to proceed with cytoreductive surgery in patients with recurrent colorectal cancer demands the removal of all cancer tissue; only this criteria qualifies the patient as potentially curable. In addition the CEA must return to a baseline value within 1 month of surgery.

Patients and Methods

Eligible patients had recurrent colorectal cancer that was positively targeted with the radiolabeled monoclonal antibody (MAb) so that the Radioimmunoguided Surgery (RIGS) protocol system could be used. All patients entered into this study underwent an extensive preoperative evaluation to eliminate, when possible, those patients with extra-abdominal recurrent tumor (*i.e.*, chest recurrence). Preoperative evaluation included CT scans of the chest, abdomen, and pelvis and bone scan when deemed necessary. Carcinoembryonic antigen tests and colonoscopy were also part of the preoperative evaluation. All patients signed an institutional review board written consent. Two days before the administration of the antibody, the patient began treatment with an oral solution of potassium iodide (SSKI, 10 drops twice a day, or KI, 500 mg twice a day) to block uptake of the radiolabeled MAb by the thyroid, and this regimen continued for 3 weeks or until surgery. Skin testing was performed before the injection. No reactions were observed. The B72.3 MAb was radioiodinated using the IODOGEN method performed in the nuclear pharmacy at The Ohio State University Medical Center. Once the negative skin test with unlabeled antibody was confirmed, the patient received, by intravenous push administration, 1 mg of the B72.3 MAb labeled with 2 mCi of iodine-125 diluted in 4 mL phosphate-buffered saline and infused for 5 minutes. All patients were observed after injection and vital signs were recorded every 15 minutes for 1 hour. Patients were scheduled for a second-look procedure when the precordial counts determined by the gamma-detecting probe (GDP), Neoprobe 1000 instrument were less than 20 counts per 2 seconds. Serum samples were obtained before injection and at surgery.

The operative procedure involved a thorough laparotomy. A formal site-by-site chart was followed to ensure complete delineation of clinically apparent sites of cancer. Following this traditional exploration, the surgeon stated his or her findings and planned procedure (*i.e.*, planned to resect all tumor or determined that the extent of tumor precluded resection and deemed the patient unresectable). Following this traditional exploration, the patient underwent detailed surveying of all potential tumor sites with the Neoprobe 1000 instrument. Two-second counts were obtained from all suspicious or preselected sites and recorded. The microprocessor-generated audio signal of the GDP facilitated the detection of areas of increased radioactivity. The background radioactivity was squelched by placing the probe over the aorta to take a background count and having the recording nurse squelch the system at this count. The GDP was then ready to produce an audible signal only when the counts exceeded the background activity by a statistically significant amount. These calculations are done automatically by the RIGS system. Biopsy specimens were taken from all sites of suspicious tissues found by palpation or inspection or in areas of increased GDP counts but in which there was no obvious tumor. These samples were then sent for frozen-section analysis. Based on clinical and GDP-directed biopsy data, a decision was made concerning the extent of the surgical procedure. In patients who underwent resective (curative) procedures, the probe was used to ascertain completeness of the resection by determining counts from the 'tumor bed' following presumed clinically complete tumor extirpation. In addition to routine histologic examination, specimens were counted *ex vivo* with the GDP and *in vitro* by well scintillation, and a panel of immunohistochemical staining was performed.

Results

Eighty-six patients were evaluated for positive identification of tumor by the RIGS system during the years 1986 to 1989. Two-, three-, four-, and five-year survival data have been gathered to compare resectable patients (n = 40), traditionally determined nonresectable patients (n = 33), and RIGS-determined nonresectable patients (n = 13). Of the 40 evaluable patients in the resectable group, 33 are alive (83%) compared to 21% and 31% of the patients in the traditionally nonresectable and the RIGS nonresectable groups, respectively.

Figure 1 depicts the sites of tumor within the resectable group and shows the number of patients alive and dead for each site. Twenty-two of the twenty-four patients who underwent major liver resection are alive (Fig. 2). The two deaths were at 1 and 2 years after the hepatic resection; the patient who died at 1 year did so of multiple bilateral pulmonary metastases found early after the hepatic resection (chest film was negative, but 3 months after operation CT of the chest showed small, diffuse, bilateral involvement of the lungs). The other patient experienced recurrence both intrahepatically and extraheptically and died 2 years later despite multiple systemic chemotherapeutic treatment. Figure 2 shows that 10 patients who had positive periportal/common hepatic artery lymph nodes, as determined by the RIGS system, were alive 2 to 5 years after hepatic resection and after total removal from the gastrohepatic area of RIGS-positive tissues. Hematoxylin and eosin, immunohistochemical staining, cytokeratin staining, and flow cytometry were performed; these analyses will be reported separately. The importance of the RIGS-positive tissues cannot be overstated. Most

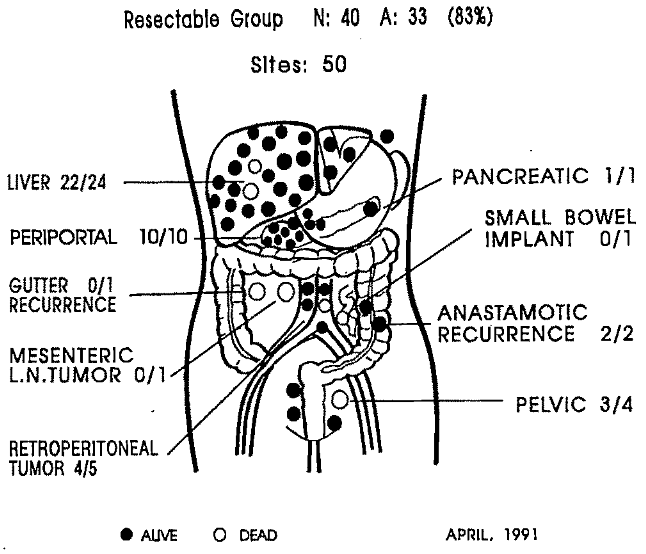

FIG. 1. Two-, three-, four-, and five-year survival rates in resectable RIGS-positive second-look patients (1986–1989).

of this tissue did not initially appear to be positive to the operating surgeon. If the RIGS-positive tissue could not be cytoreduced completely, these patients were placed in the nonresectable group. The planned major hepatic resection or placement for regional chemotherapy catheters was aborted, and treatment by systemic chemotherapy was chosen instead.

The second most common resectable recurrence is retroperitoneal tumor, and Figure 3 depicts these data. Included in the diagram are five retroperitoneal, infrapancreatic tumor deposits; four of these five patients are alive. The only death occurred 1 year after the retroperitoneal dissection. The retroperitoneal dissection started in the pelvis and continued up to and beneath the renal veins and ended further up into the celiac area. It was aortic and retrocaval. Had the cephalad tumor been assessed earlier in the procedure, this patient would have been deemed unresectable. One patient with recurrence in the distal pancreas appeared in this group and continues to do well 5 years later. The most cephalad site was above and posterior to the spleen, and a second nodule was found with the GDP after complete removal of the first nodule cephalad to the diaphragm in the left chest. The diaphragm was opened and the second nodule removed. The patient's death 2 years after surgery resulted from a right gutter recurrence. Additional tumor was probably missed at the second-look surgery and diffuse retrocaval tumor was found on a third exploration. As a result the malignancy in this patient was deemed too extensive to resect. The last patient in this group died 4 years after second-look surgery. He had mesentery and superior mesenteric artery tumor encasement that had been meticulously dissected during the surgery. Further tumor recurred in the liver 2 years after second-look surgery and was removed, but ultimately there was bilateral lung recurrence 3 years after second-look surgery.

There were two patients with anastomotic recurrence and one patient with small bowel implant who underwent

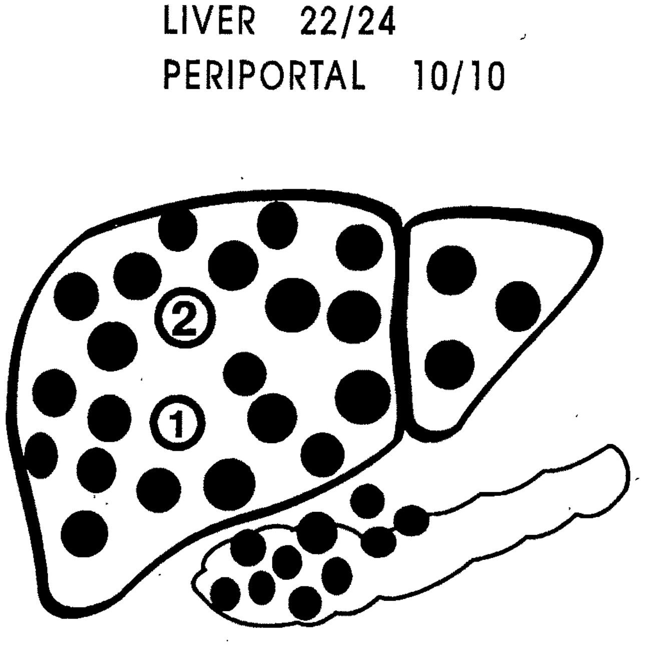

FIG. 2. Liver and periportal recurrences in the resectable group.

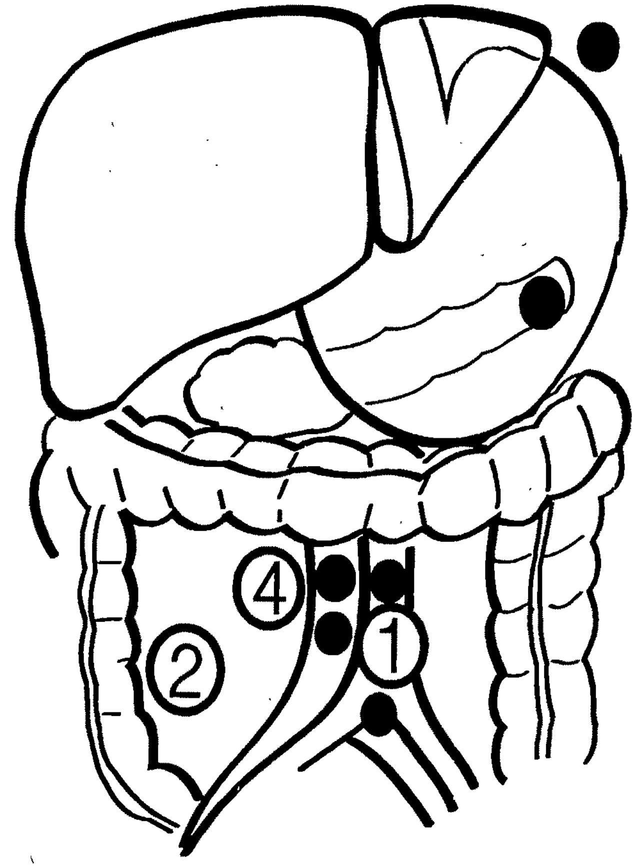

FIG. 3. Retroperitoneal recurrence in the resectable group.

curative resections. The only death involved the patient who had a small bowel mesenteric implant removed. He died of a massive coronary attack 2 years after second-look surgery with no evidence of disease (NED) (Fig. 1). The last patients analyzed in the resectable group were those with pelvic recurrence. Three of the four remain alive (NED) and the fourth died 4 years after pelvic *en bloc* removal of the recurrent tumor (Fig. 4).

A comparison of the three groups, resectable, traditional nonresectable, and RIGS nonresectable, shows major differences at yearly intervals (Fig. 5) Probability values comparing survival by Lee-Desu statistical analysis are resectable *versus* traditional nonresectable, $p < 0.0001$; resectable *versus* RIGS nonresectable, $p = 0.0008$; and traditional nonresectable *versus* RIGS nonresectable, $p = 0.24$ (not significant). The median survival is 60+ months for the resectable group, 18 months for the traditional nonresectable, and 29.4 months for the RIGS nonresectable. The 2-year survival rates were 95%, 36%, and 57%, respectively; 3-year survival rates were 83%, 7%, and 37%; and 5-year survival rates were 60%, 0%, and 0%.

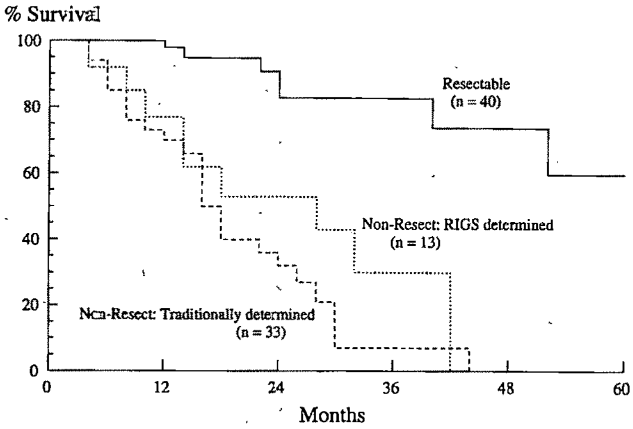

FIG. 5. Survival in RIGS-positive patients.

Discussion

Second-look surgery has been studied for 40 years, since 1951 when Wangensteen and colleagues first described the concept and operated on patients at preselected time intervals (6 months at first and then 9 months) following potentially curative resection for colorectal cancer. While the idea was good, only a few patients benefited (6.2% 5-year survival rate). Wagensteen's original hypothesis stated that the factor that might influence most favorably the ultimate result of cancer surgery was the absence of cancer in all regional lymph nodes. Fear of having left behind residual cancer prompted Wangensteen to propose a second operation to re-enter the abdomen before the expiration of the 'silent' interval. Selecting the most appropriate time for second-look surgery presents a challenge. In theory, by the time the second-look operation is done, any residual local cancer should have grown large enough to be detected but still be small enough to be completely removed. Between 6 and 14 months after surgery was the interval Wangensteen reported at which the patients with recurrence still had resectable tumor. But variability in tumor growth rates continues to be a problem in determining when to perform second-look operations.

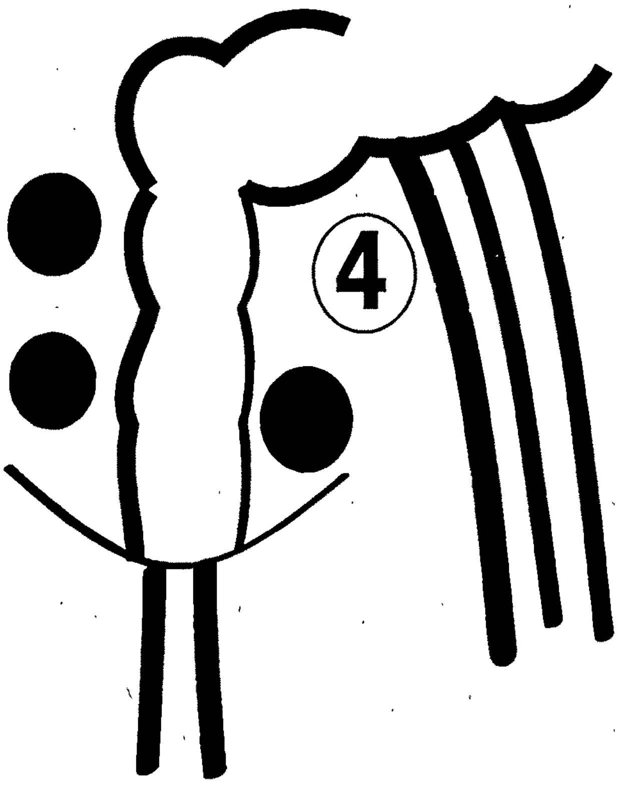

FIG. 4. Pelvic recurrence in the resectable group.

In 1954 Wangensteen reported 46 operations for 35 patients with cancer of the rectum; no cancer was found in 20; 13 others remained alive; of these 13, 5 were living with cancer; and 2 patients died of cancer.[2] Of the 15 patients found to have cancer at the second-look operation, one was living 12 months after the last negative second look, 11 were dead of cancer, one died at operation, and one was alive with residual tumor while awaiting a third look. Poor results were thought to be caused by the anatomic restrictions in the pelvic area and the inability to remove all cancer. On the other hand, the 1954 report concerning colon cancer patients was more encouraging. Twenty-nine patients underwent a total of 45 operations. No cancer was found at second-look in 15 patients. Of the 14 patients who had a positive second-look procedure, four were alive and free of tumor, six died from cancer, three operative deaths occurred, and one patient was living with residual cancer. The report argued convincingly that a biologic tumor marker would be helpful in decreasing negative second-look operations.

In 1985 we reported that the biologic marker CEA greatly improved the number of positive abdominal entries and essentially eliminated negative second-look procedures.[7] Of the 146 second-look procedures, 139 were positive. The 5-year survival rate in this group was 23.9%.

A 1987 report by Chu and colleagues[8] indicated that of 21 patients who underwent CEA-directed, second-look laparotomy (LAP), 63% were resectable, whereas only 33% of 48 patients who undergone clinically directed second-look laparotomy were resectable. Two of the CEA-LAP group were negative for recurrent tumor. Patients with resected lesions showed a 5-year survival rate of 22%, while there were no survivors at 5 years for nonresected patients. A subgroup of CEA-LAP patients had a 5-year survival rate of 48%, which supported the concept that earlier (asymptomatic) second-look procedures are beneficial.

Further support for the surgical treatment of recurrent colorectal cancer was reported in 1989 by Makela et al.,[9] who concluded that when possible, resective surgery improved survival. They also concluded that recurrent colorectal cancer is caused by a failure to remove all of the original tumor and that the recurrence happens within 2 years. Their operative mortality rate was 13% of resectable patients. They concluded that intensive follow-up can lead to earlier detection of recurrent colorectal cancer and an increased successful resection rate for cure. The results of this study support improved survival through more radical reresections.

As better survival rates are attained with less incidence of morbidity and death, the ability to select the candidates for major surgical procedures has become increasingly important. The continuing argument concerning resection of intrahepatic and extrahepatic tumor needs to be addressed. The liver is the major site of recurrence (Figs. 1 and 2). The ability to choose which patients should undergo major hepatic resections has improved with better preoperative selective criteria and with newer intraoperative techniques that provide much more information than traditional methods about less obvious tumor deposits. The newer retractors have permitted better operative exposure and both the intraoperative ultrasound probe and the handheld, gamma-detecting probe have increased the ability to find occult tumor. Finally the CUSA (an ultrasonic dissector) has enabled surgeons to do more anatomic resections and has decreased the chances of cutting across pseudopods of tumor tissue. It has also allowed better visualization of tumors, including the less obvious satellite lesions. The gamma-detecting probe has allowed better assessment of the completeness of resection by checking the bed of the tumor after tumor removal. Deeper lesions, less obvious lesions in the caudate lobe and near the vena cava, and surgical margins have all been better identified and addressed with these newer technologic advances.

Extrahepatic tumor deposits in less obvious sites are being identified with increasing frequency by the handheld, gamma-detecting probe. Celiac, infrapancreatic, retrocaval, retroaortic, and deep pelvic tumor deposits are also being identified with increasing frequency by the GDP, necessitating more involved lymph node sampling and removal.[10] Of the technologic advances, the RIGS system is especially outstanding in assessing lymph nodes and identifying, with increasing frequency, this extrahepatic tumor that would not be found by traditional means.

As the extrahepatic RIGS-positive tissue is evaluated at successive stages, a carefully planned curative resection can be more accurately performed. In our study traditional methods of palpation and inspection deemed 62% of patients undergoing second-look procedures as resectable, but the RIGS system identified only 47% as good candidates for curative resection. The new RIGS technology helped the surgeon to be more precise in the selection process. The overall survival rate for the more precisely selected resectable group is 83% still alive *versus* 21% for the traditional nonresectable group, and 31% for the RIGS nonresectable group. The 2-year survival rates were 95%, 36%, and 57%, respectively, with 3-year survival rates of 85%, 7% and 37%, and 5-year survival rate of 60%, 0%, and 0%.

Acknowledgments

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DISCUSSION

DR. PAUL H. SUGARBAKER (Washington, DC): Drs. Martin and Carey remind us the results of surgery are only as good as our ability to exclude elsewhere disease in patients who are to undergo a potentially curative resection of cancer. One of our major problems, however, in assessing radiologic studies in patients with liver metastases is that we cannot seem to find an ultimate test.

And as we study computed tomography (CT), ultrasound, CT portogram, magnetic resonance imaging (MRI), and now the RIGS System, we find that all of these studies show us certain patients who benefit from additional knowledge.

And so what I would like to ask Dr. Martin as my first question is, how does the RIGS System compare with the intraoperative ultrasound in finding additional lesions within the liver in patients undergoing surgery in hopes of resecting liver metastases?

Then I would like to suggest to him that perhaps there is a second valuable usage of the RIGS System. In our patients with peritoneal carcinomatosis, we have the challenge of trying to remove the last cancer cell from the abdominal cavity, which we all know contains virtually thousands of nooks and crannies. As we attempt to remove the last mucinous tumor cell from this large surface area, we find that the surgeon's eyes are sometimes not what we would hope they would be. And Ted, we have found that in our patients it is not unusual for us to scan the operative field with the RIGS instrument and find out that there is a small foci of residual disease in the pelvis or up underneath the liver in Morrison's pouch, or in and along the hepatogastric ligament.

And so my comment is that we have found that the RIGS System is of considerable value in assessing the completeness of a surgical resection for colonic adenocarcinoma.

DR. JOHN M. DALY (Philadelphia, Pennsylvania): Seventy-five thousand deaths will occur in the United States this year from large bowel cancer. The statistics are really sobering. Some argue for very intensive follow-up surveillance for patients after the primary resection, and yet others argue that patient worry, health care costs, and questionable benefit should allow us to use a more laissez-faire attitude.

Monitoring carcinoembryonic antigen (CEA) levels in plasma and more recently in bile has proven to be sensitive for detecting tumor recurrence before clinical signs and symptoms develop.

The major question really is, does earlier detection truly result in greater cure rates? It is hoped that the current English trial will help to answer that question.

The use of monoclonal antibodies is clearly one approach to tumor detection. The intraoperative probe discussed by Dr. Martin allows precise sampling of certain areas within the abdomen. We have used the B72.3 antibody with either iodine or indium as the primary radioisotope. Using the RIGS technique and studying 14 patients, we have seen a true positivity rate in about three quarters of the patients studied, whereas, because the indium is taken up so avidly by the Kupffer cells, only about one in five patients had a true positive study. We saw false positives in a little less than 10% in both groups.

I have several questions for Dr. Martin. Thirteen patients were determined to be unresectable by RIGS but resectable by traditional approaches of exploration. What areas did the surgeons miss the most?

Were there any false positives using the RIGS technique?

And finally in several series hepatic resection has not been shown to provide cure if extrahepatic lymph nodes, particularly in the periportal area, are involved with tumor. Yet in your series, 10 of 10 patients are alive 2 to 5 years after the combined hepatic resection and lymph node dissection. Are they alive without disease, and could you comment on this?

I enjoyed the paper very much, and I think it is a major piece of work. Thank you.

DR. HARRY WANEBO (Providence, Rhode Island): This is an interesting presentation this morning, because the authors describe the use of a technique using a radiolabeled monoclonal antibody to identify cancer-containing tissue, which otherwise might be occult to surgical detection by palpation or visualization.

Among the 86 patients evaluated, there were 53 (62%) deemed resectable by traditional exploration, but only 40 were considered resectable by the RIGS. There were 13 potentially resectable who were thus excluded. The survival rate of these 40 patients was 60%. Now if the 13 had not been excluded and had been resected or the effort had been made, the survival rate would then be approaching what we normally obtain, which is around the mid-40% range for CEA second-look procedures. Thus the RIGS appears to upstage these patients, and eliminates those who would not benefit from the surgery.

Obviously it will have to be determined by randomized trial whether this truly makes a difference, and I know a trial is under way comparing the traditional approach with the traditional plus this technique.

There are some questions, however. One of these, I think, was alluded to by John Daly. For example, the finding and resecting of tissue that is positive in the patient's periportal area was associated with an amazing survival rate. Personally I have not seen this myself where patients have liver metastases and periportal disease. Usually we consider those patients nonresectable.

So I would ask the authors whether this tissue contained cancer, or whether or not there were just macrophages containing the monoclonal antibody.

Dr. Daly showed us data regarding sensitivity and specificity, but I would ask, Ted, what is the sensitivity and specificity among your own patients? Obviously the RIGS does not identify every cancer nodule. I presume it is around 80%. So there is going to be a misinterpretation. Obviously it is going to take both the traditional technique and the use of RIGS to actually determine the maximum number of nodules within the patient.

Lastly there are some sites that are very difficult to deal with, either where tumors are invading the pelvis or are invading vascular structures. How useful is the RIGS in these areas? Will you pursue resection and then can RIGS really determine that you are disease free?

DR. BLAKE CADY (Boston, Massachusetts): I too had the privilege of looking up Dr. Wangensteen's original articles and all the follow-up, and it was interesting, although it was not mentioned publicly, that in these articles there were patients who presented with symptoms at the same time as he had patients who were asymptomatic, and the survival in both groups was the same.

As a matter of fact, in a 1985 publication of Martin and Minton, the survival curve of the CEA-determined reoperative group and the non-CEA or symptomatically determined group was exactly the same.

I am always concerned about trying to change a biologic phenomenon by temporal speed-up, and I just want to ask the authors, isn't this just another phenomenon of lead time bias, the speeding up of finding occult metastases, that ultimately will not change the outcome?

DR. EDWARD W. MARTIN, JR. (Closing discussion): I think I will address Dr. Cady's question first, because it is freshest in my mind. We are using a biologic tool, the monoclonal antibody, to deal with a biologic disease. The radiolabeled monoclonal antibodies have no emotions, and sometimes they are very cruel. You think that you have completed your surgical procedure, but by using this biologic marker, you have more work to do. So the RIGS system has both lengthened and shortened surgeries. This system is a biologic tool that we are dealing with and this addresses the biologic behavior of this disease.

I agree with Dr. Cady 100%, that we do not completely understand the biology of any of the cancers, but we are doing better.

As far as Dr. Sugarbaker's comments, I think that the ultrasound probe is an adjunct to the gamma-detecting probe. The ultrasound probe finds deep lesions in the liver that are often difficult to palpate. Both probes really work together; they work in concert, not one better than the other.

As far as Dr. Daly's comments, I am interested in his CEAs in the bile. We have not been studying the bile. One wonders how Dr. Daly gets bile to determine recurrence noninvasively.

As far as the periportal area is concerned, I made that slide knowing that it was going to create an entire discussion in itself. You must realize that the periportal area is full of RIGS-positive tissue. If I were to make one suggestion to anybody who does not have a probe, I would encourage them to perform a dissection in the gastrohepatic area as part of any liver resection. This would be comparable to doing the axillary dissection for breast cancer. It is worth addressing even though you do not have a probe to help you to decide if there is tumor, because about 50% of the time there will be extrahepatic tumor in this area that is not obvious to palpation or inspection. One must remember that we often fail extrahepatically after completing a major liver resection.

With regard to the lymph nodes, I think the pathologists will quickly catch up with the new technology and begin to further examine these nodes with more innovative techniques. Our lymph nodes in the RIGS project are sent immediately to different laboratories. As newer techniques are completed, more and more of these lymph nodes prove to be involved with microscopic tumor, and these nodes indicate a bad prognosis. Even though it is difficult to find small deposits of tumor cells with a light microscope, with newer laboratory techniques (flow cytometry, special immunohistochemical staining, antibody sorting, etc.) we are beginning to improve our understanding of RIGS-positive nodes. Much of the information may be at a premorphologic level (cytokines, monokines, and mucins).

The Role of Multimodality Therapy in Soft-tissue Sarcoma

MURRAY F. BRENNAN, M.D.,* EPHRAIM S. CASPER, M.D.,† LOUIS B. HARRISON, M.D.,‡ MAN H. SHIU, M.D.,*
JEFFREY GAYNOR, PH.D.,§ and STEVEN I. HAJDU, M.D.||

Soft-tissue sarcomas are uncommon malignancies. The development during a period of 8 years, in one institution, of a prospective data base incorporating more than 1600 patients with these tumors is described. The most common sites for occurrence are the extremities, but they can occur in any of the soft tissues of the body. Liposarcoma and leiomyosarcoma are the most common histopathologic conditions identified. Prognostic factors for both recurrence and survival include site, histopathology, size, grade, and adequacy of resection. A prospective randomized trial of the use of adjuvant radiation by the brachytherapy technique in extremity lesions has shown a decrease in local recurrence, but no impact on survival. Eligible patients not randomized to the trial show no difference in local recurrence or survival, regardless of whether they received adjuvant radiation.

SOFT-TISSUE SARCOMAS are ubiquitous neoplasms that arise in the soft, or connective, tissues of the body. They are diverse in histology and occur at any site throughout the body. Most of them predominate in the extremities. Soft-tissue sarcomas in children, especially the most common form, rhabdomyosarcoma, have been prototypes for multimodality therapy.[1] Children previously treated only by radical and mutilating surgery can now be treated by more function-sparing procedures, chemotherapy and radiation therapy, with the chance for long-term, disease-free survival.[2] The challenge to transfer to adults the improved results seen in children has been one of frustration. The present report documents some of our experiences with these neoplasms and focuses on a recent prospective randomized trial to control local recurrence, by the addition of adjuvant radiation therapy using the brachytherapy technique, in patients with adult extremity soft-tissue tumors.

From the Departments of Surgery, Medicine,† Radiation Oncology,‡ Biostatistics,§ and Pathology,|| Memorial Sloan-Kettering Cancer Center, New York, New York*

Materials and Methods

In 1982 we began a prospective database at Memorial Sloan-Kettering Cancer Center (MSKCC) for all adult (age more than 16 years) patients admitted and treated for soft-tissue sarcoma. This database has been used for the development of prospective evaluations of the management of these tumors and the correlation of clinical and biologic parameters for outcome and survival. All patient tissue biopsies and tumor resections have been reviewed by a single pathologist. All patients have been reviewed and staged on a weekly basis as to extent of disease, and multiple demographic features have been recorded. These include factors known to influence outcome, such as age; sex; tumor site, size, depth, histopathologic grade, and type; and presence or absence of metastasis.

A prospective randomized study of the benefit of brachytherapy (BRT) was begun in July 1982 and is reported here as of closure in July 1987. Patients were ineligible if they had received previous radiation therapy or chemotherapy or if they were foreign nationals, for whom long-term follow-up might be difficult. All patients with localized extremity or superficial truncal lesions were considered eligible, providing they had a limb-sparing operation that would completely remove all gross disease without tumor violation and without the need for resection of major vessels, nerves, or bone. Patients were included if they presented either with a primary untreated but biopsied lesion, or with a locally recurrent but nonmetastatic tumor. All patients had a tissue diagnosis of soft-tissue sarcoma before entrance into the trial.

Signed informed consent was obtained before operation and intraoperative randomization was performed once

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Address reprint requests to Murray F. Brennan, M.D., Chairman, Department of Surgery, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021.

we could determine that all gross tumor could be resected. Tumor violation was an intraoperative exclusion, as was the failure to remove the tumor completely as judged by the operating surgeon. Extracapsular excision, in which a defined gross margin of normal tissue could not be obtained, was acceptable. Stratification was used before randomization for known prognostic variables previously identified.[3,4] These included tumor presentation (primary or recurrent), tumor size (less than or more than 5 cm), tumor depth (superficial or deep), and tumor grade (high or low). Once randomized, afterloading catheters were placed in the tumor bed, and on the fifth to sixth postoperative day, these catheters were loaded with Iridium-192 sources. A total dose of approximately 4200 to 4500 cGy was delivered in 4 to 6 days to the isodose contour that incorporated the target volume determined at the time of operation.[5,6] Patients with high-grade tumors were eligible to receive postoperative chemotherapy as part of a further randomized trial of the benefits of adjuvant chemotherapy, with Adriamycin (Adria Laboratories, Dublin, OH) given by either the bolus or continuous-infusion technique.[7] Patient follow-up examinations were performed every 3 months for the first 3 years, then every 6 months thereafter. All nonrandomized patients were followed in the same manner as those who were randomized. The margin of resection of any tumor situated within 1 mm of an inked surface margin of the surgical specimen was considered microscopically positive.

Statistical evaluation includes the use of chi square, Kaplan–Meier survival curves, and log-rank analysis, performed where indicated.

Results

From 1982 through 1990, we admitted 1685 adults who were more than 16 years old with soft-tissue sarcoma. Most tumors (50%) occurred in the extremities (Fig. 1). The most common histopathologic diagnosis was liposarcoma, followed by leiomyosarcoma and malignant fibrous histiocytoma (MFH) (Fig. 2). In the extremities and superficial trunk, liposarcoma and MFH were most common, whereas in the retroperitoneum and visceral tissues, leiomyosarcoma predominated. Survival rate based solely on site and individual histopathology are shown in Figures 3 and 4. Because tumor grade and site are so important, we documented outcome based on high-grade status alone within a single site, the extremity (Fig. 5).

Between 1982 and 1987, 997 patients were admitted to MSKCC; of 225 eligible patients, 126 were randomized in the trial of BRT. Failure to randomize was due primarily to patient refusal to accept entrance into an 'ex-

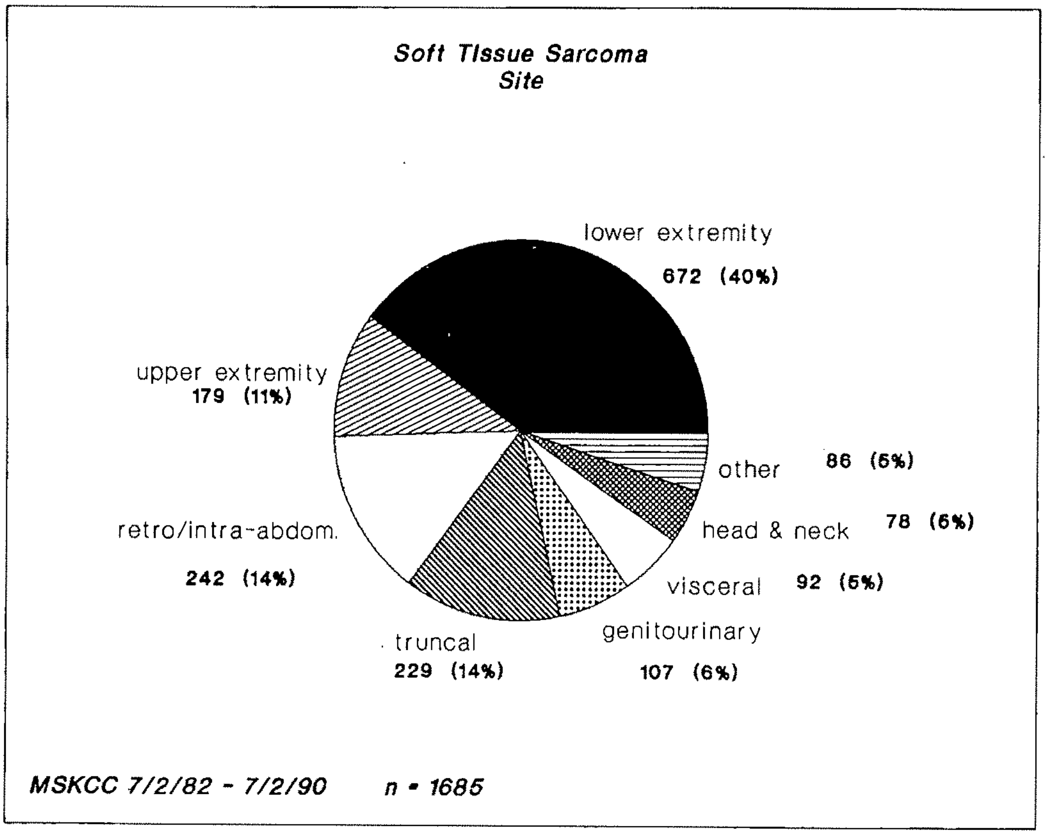

FIG. 1. Site of sarcoma in 1685 patients admitted from 1982 to 1990.

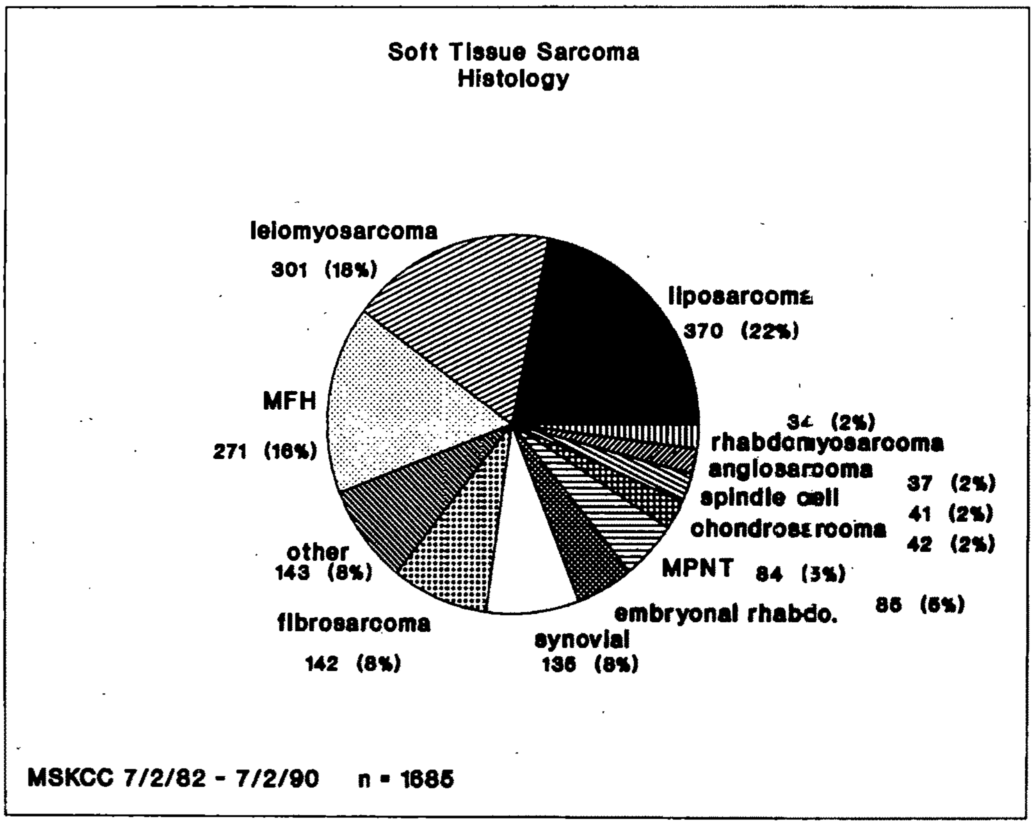

FIG. 2. Histopathology of soft-tissue sarcoma for 1685 patients with soft-tissue sarcoma, admitted from 1982 to 1990.

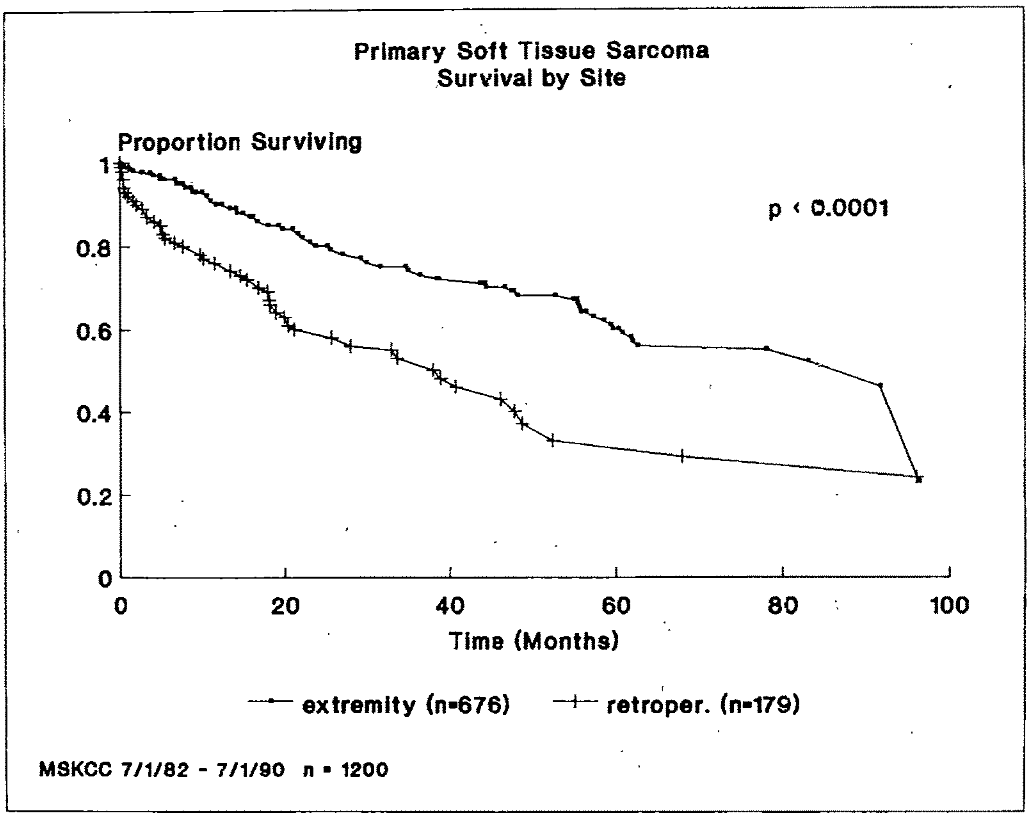

FIG. 3. Survival by site: extremity lesions have a better overall prognosis than retroperitoneal lesions ($p < 0.0001$) for patients presenting with localized disease, for whom complete follow-up is available.

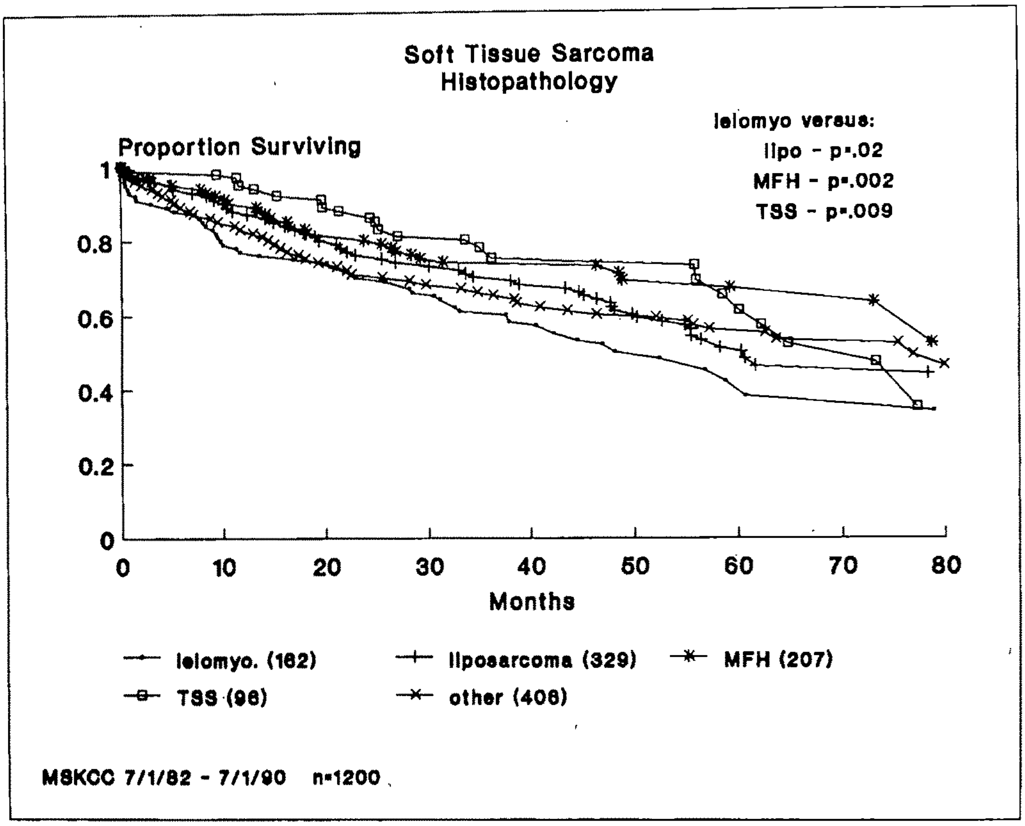

FIG. 4. Survival by histopathology for patients with primary (nonmetastatic) tumors at presentation.

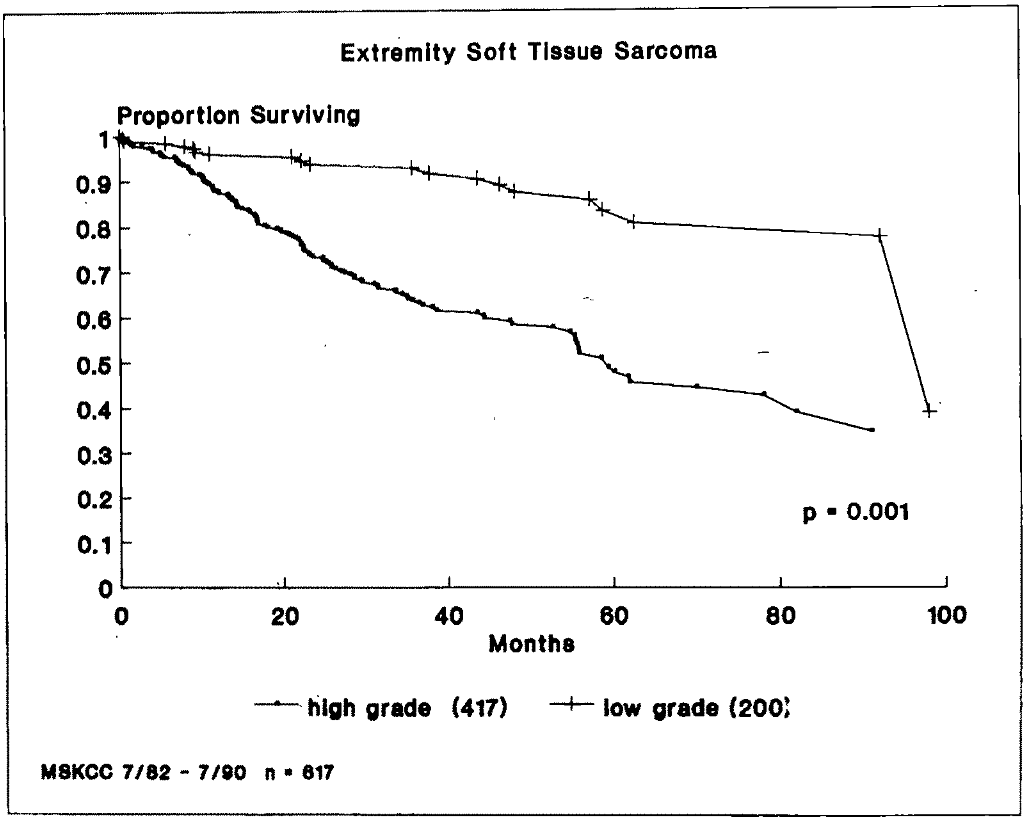

FIG. 5. Extremity soft-tissue sarcoma. Patients with primary localized lesions at presentation. Influence of grade is shown.

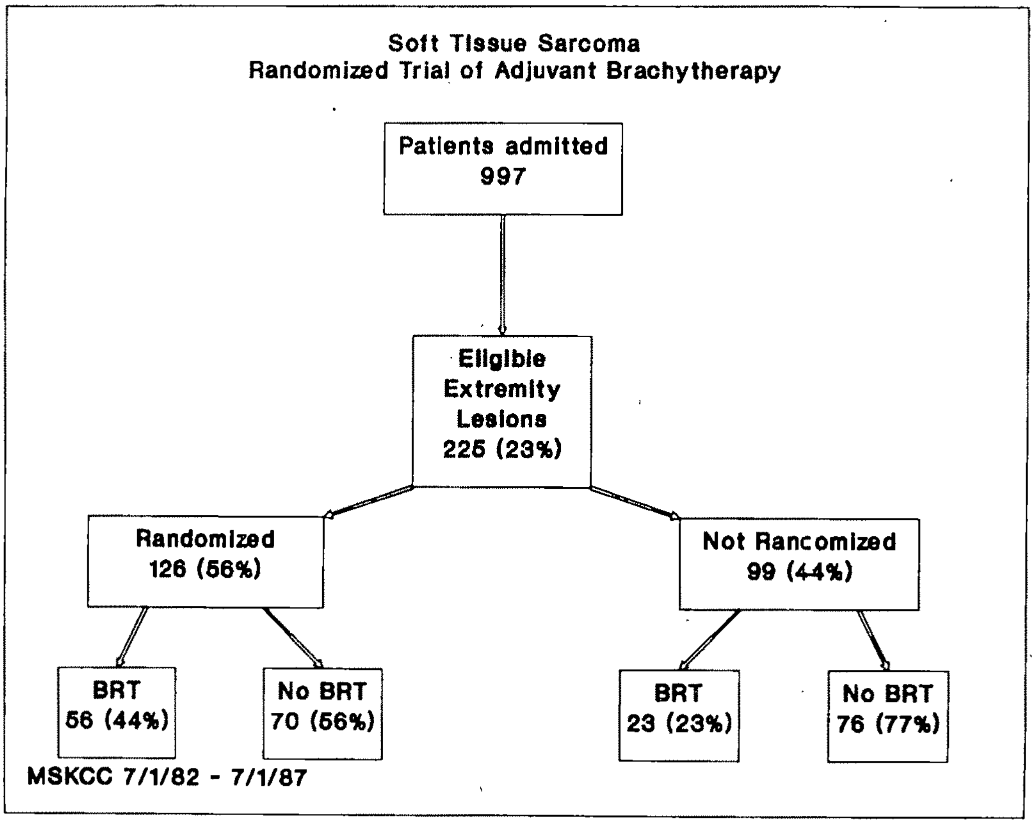

FIG. 6. Schematic showing randomization of patients for adjuvant brachytherapy.

periment.' Of those patients not randomized, approximately 25% received BRT and 75% did not (Fig. 6). Follow-up data dates from March 1, 1991, with a median of 40.75 months for all patients, and 46.5 months for survivors.

The appropriateness of our preoperative stratifications are indicated in Table 1, showing patients equally distributed between the two groups according to risk factors. The differences between the numbers entered for the two groups was a consequence of our initial expectation that we would need 250 patients to show effectiveness, and because of multiple prerandomization stratifications, group allocation was performed such that a balance for each stratum would be achieved when 30 patients were entered into each stratum. The fact that 30 patients were not entered into every stratum at the time of closure confirmed the appropriateness of the stratification strategy but resulted in a slight disparity in the total number of patients in each group. The patients were equally distributed by histopathologic diagnosis (Table 2). There were no differences within the two groups between patients with high-grade lesions who received adjuvant Adriamycin, and no difference in the number with positive microscopic margins (Table 3). As expected, nonrandomized patients who received BRT had a greater proportion of large, deep, high-grade lesions than those not receiving BRT (Table 4).

The results of the impact on local recurrence are illustrated in Figure 7, in which any local recurrence is demonstrated to be diminished by the addition of BRT. This is true also when local-only recurrence is examined (Fig. 8). No effect on survival has yet been demonstrated (Fig. 9). Interestingly subset analysis, analyzing only high-grade

TABLE 1. *Soft-tissue Sarcoma: Randomized Trial of Adjuvant Brachytherapy*

Patient and Tumor Characteristics		BRT (n = 56)		no BRT (n = 70)	
Age (years)	>50	33	59%	39	56%
	<=50	23	41%	31	44%
Sex	Female	25	45%	24	34%
	Male	31	55%	46	66%
Site	Proximal	50	89%	59	84%
	Distal	6	11%	11	16%
Initial size	>5 cm	40	71%	52	74%
	<=5 cm	16	29%	18	26%
Depth	Superficial	24	43%	21	30%
	Deep	32	57%	49	70%
Grade	High	45	80%	52	74%
	Low	11	20%	18	26%
Presentation	Primary	50	89%	60	86%
	Recurrent	6	11%	10	14%

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All not significant by chi square test.
Patients are equally distributed according to risk factors.

TABLE 2. *Soft-tissue Sarcoma: Randomized Trial of Adjuvant Brachytherapy*

	Histopathology	
	BRT (n = 56)	no BRT (n = 70)
Liposarcoma	22 (39%)	27 (39%)
MFH	13 (23%)	17 (24%)
MPNT	6 (11%)	3 (4%)
Synovial	4 (7%)	4 (6%)
Leiomyosarcoma	4 (7%)	6 (9%)
Other	7 (13%)	13 (19%)

Patients are randomized by histopathology.

MFH, malignant fibrous histiocytoma; MPNT, malignant peripheral nerve tumor.

TABLE 4. *Soft-tissue Sarcoma: Nonrandomized Patients*

Characteristic		BRT (n = 23)		no BRT (n = 76)	
Age	>50	9	39%	26	34%
	<=50	14	61%	50	66%
Sex	Female	12	52%	32	42%
	Male	11	48%	44	58%
Site	Proximal	20	87%	55	72%
	Distal	3	13%	21	28%
Initial size	>5 cm	13	56%	15	20%
	<=5 cm	10	44%	61	80%
Depth	Superficial	4	17%	48	63%
	Deep	19	83%	28	37%
Grade	High	16	70%	42	55%
	Low	7	30%	34	45%
Presentation	Primary	19	83%	60	79%
	Recurrent	4	17%	16	21%

MSKCC 7/82–7/87.

lesions, suggests that the effect is due to the impact on local recurrence in high-grade lesions alone. Currently the benefit of adjuvant BRT for low-grade lesions has not been demonstrated, although the numbers are small. Interestingly one would expect the high-grade lesions to be accompanied by subsequent metastatic disease, yet the impact on long-term survival has not been demonstrated. This important observation is addressed further in the Discussion.

When we examine the characteristics of patients who did have a local recurrence as the first demonstration of recurrence, there was no difference in the distribution by risk factors. Microscopically positive margins did not appear to be a factor. Nonrandomized patients showed no apparent benefit from BRT (Fig. 10), which emphasizes the importance of randomized trials. Of 22 patients with positive microscopic margins (Table 3), three in the BRT group experienced local recurrence, as did three patients in the non-BRT group. Equal numbers of patients received or did not receive Adriamycin in the groups that experienced local recurrence.

Discussion

Several major questions exist about the management of soft-tissue sarcoma. Some address the entire biology of the management of malignancy. The issue of whether more conservative, *i.e.*, less radical, surgery can be performed and preserve limb function without sacrificing long-term freedom from local and distant recurrence is an important biologic concept. It has been addressed primarily in the management of breast carcinoma. However, to show that control of the local lesion can affect long-term survival, which many believe is predetermined, requires that a randomized trial can demonstrate decreased local recurrence by the use of an adjuvant modality. Once this is established in a trial setting, follow-up data on patients with diminished local recurrence can be examined in terms of survival. The current study addresses this issue. It would appear that at the present time, decrease in local recurrence is not accompanied by an improvement in the long-term survival rate. This important observation required continuation and long-term follow-up study.

TABLE 3. *Soft-tissue Sarcoma: Randomized Trial of Adjuvant Brachytherapy*

	BRT (n = 56)	no BRT (n = 70)
Pathologic margins		
Micropositive	11 (20%)	11 (16%)
Micronegative	45 (80%)	59 (84%)
High grade only	45 (80%)	52 (73%)
Adriamycin	34 (76%)	31 (60%)
No adriamycin	11 (24%)	21 (40%)

MSKCC 7/82–7/87.

All not significant by chi square test.

Pathologic margins and chemotherapy in each group.

Other important observations have been made as a consequence of examination of these interesting lesions, particularly as they relate to size and depth of the primary tumor in predicting survival. Previously no one group or institution has had enough patients to examine specifically outcome in terms of local recurrence or survival for individual sites or individual histopathologic diagnoses. As a consequence of the development of this data base, we have consistently tried to answer a number of questions about these patients and their tumors.

Initially, in a retrospective review of patients seen from 1968 to 78 and followed for more than 8 years, we identified factors that predicted both local recurrence and survival. These included patient age and tumor site, size, depth, histopathologic type, and histopathologic grade.[8–10] Careful dissection within the histopathologic determination, *e.g.*, liposarcoma, permitted the finding

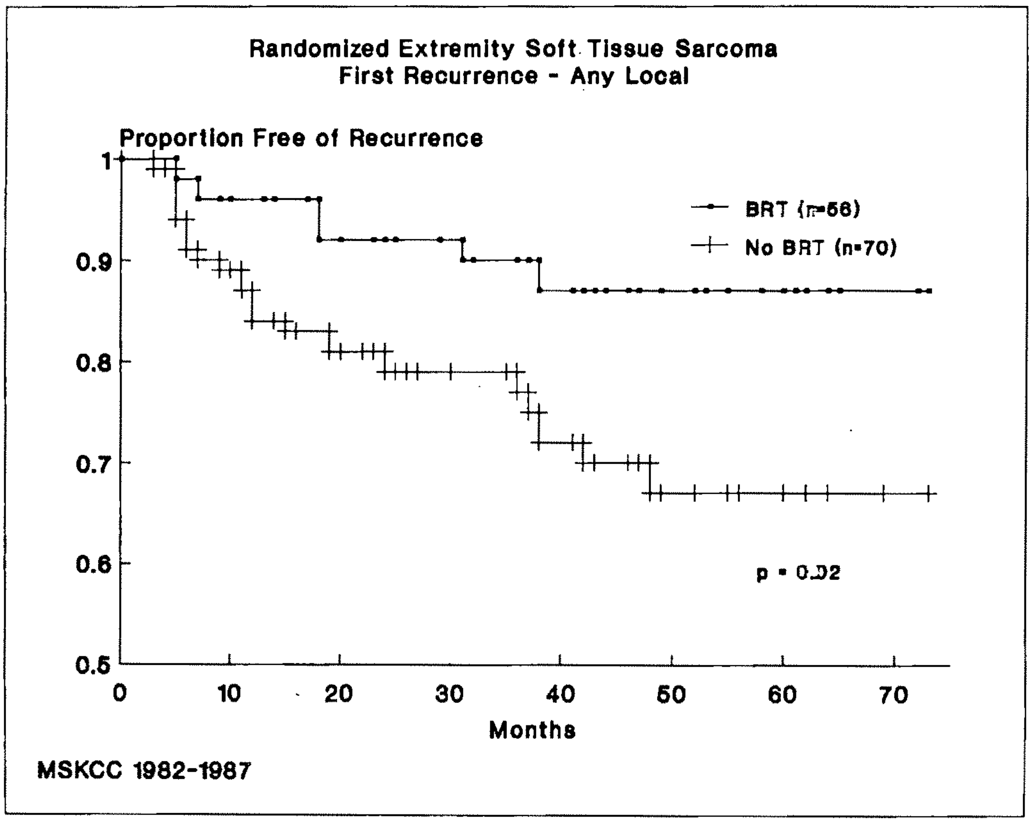

FIG. 7. Local recurrence is diminished by the addition of brachytherapy (follow-up March 1, 1991).

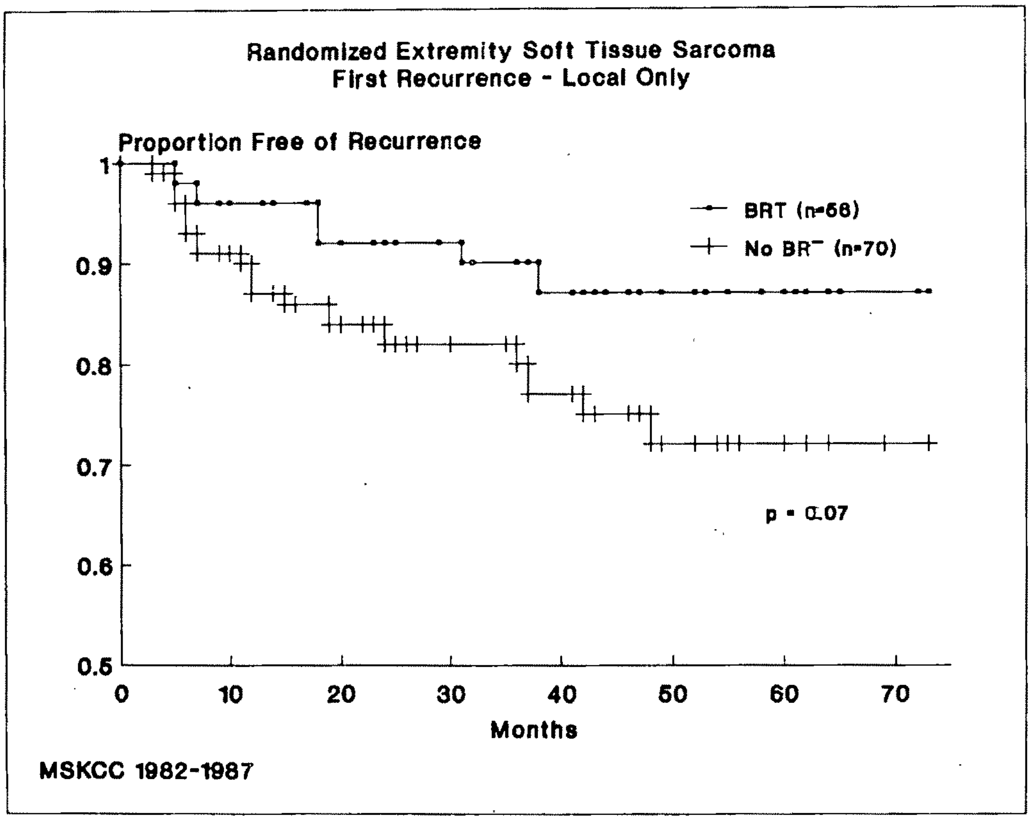

FIG. 8. Local-only recurrence is diminished by the addition of brachytherapy (follow-up March 1, 1991).

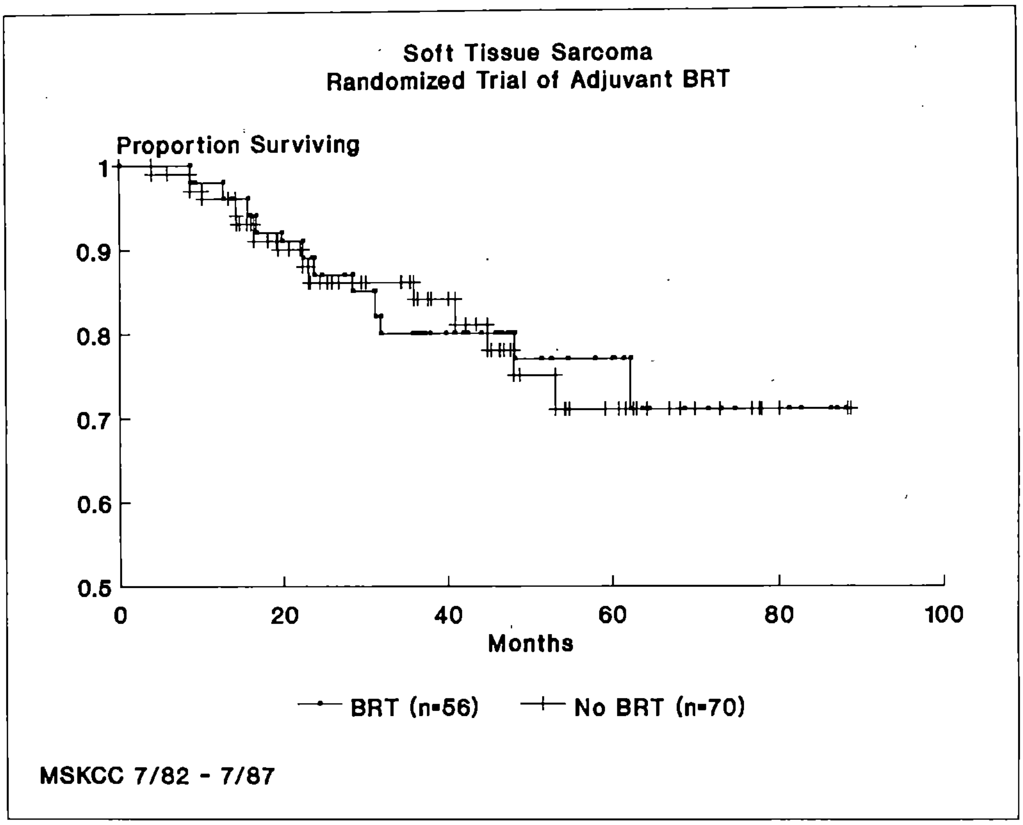

FIG. 9. No effect on survival has been demonstrated (follow-up March 1, 1991).

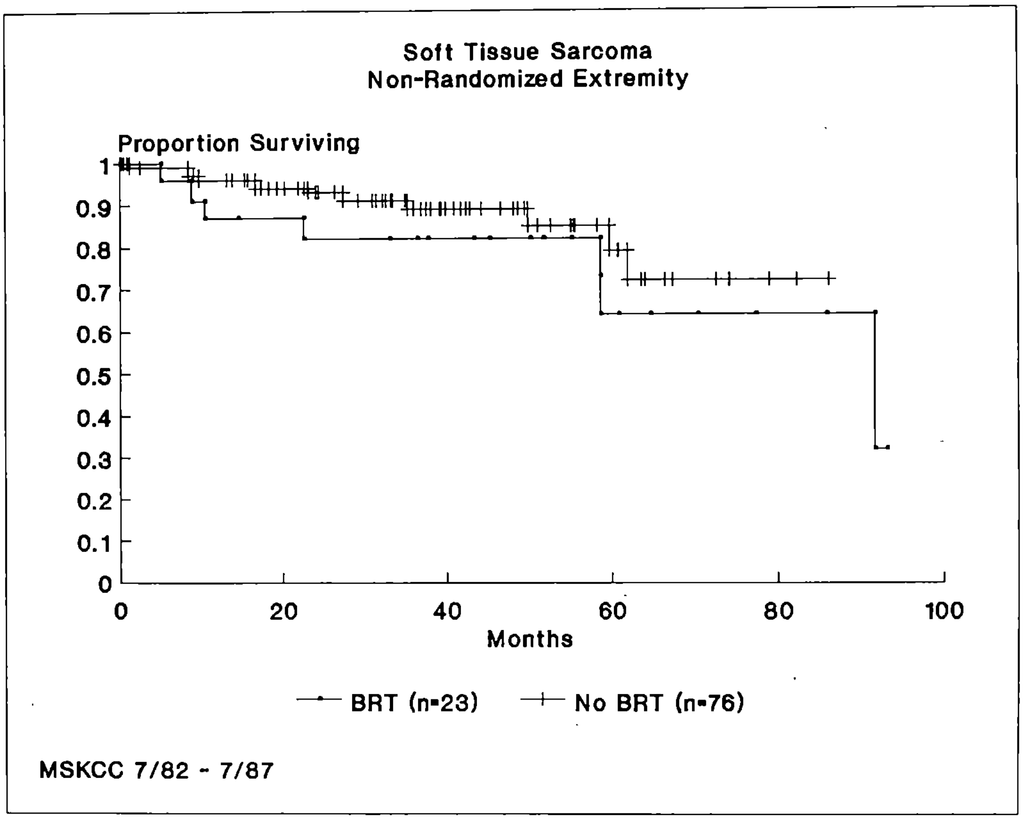

FIG. 10. Nonrandomized patients who received BRT showed no apparent benefit in effect on local recurrence.

that histologic subtype and grade could be predictors of local and systemic recurrence.[11] Careful examination of patients with low-grade lesions has allowed some insight into the possibility (less than 25%) of metastases from these lesions.[12]

Staging systems for soft-tissue sarcoma have focused on size and grade in the presence or absence of metastasis. Historically we have used tumor size and grade, as well as presence of invasion, to help characterize the potential outcome of patients. Grade has been used widely in either four levels or three. We have used only the high- or low-grade designation, a distinction that is easier to manage and circumvents the difficulty of interpreting individual histopathologic variation in what is considered a grade II or grade III lesion when three or four categories are used. With the combination of tumor size, depth, and grade, we have been able to achieve excellent separation of outcome. The only other available data are from the American Joint Commission on Cancer published some years ago by Russell et al.[13] Our definition of tumor within 1 mm of any inked margin as a positive microscopic margin exaggerates the possibility of positive microscopic margins, but it does allow consistency. Certainly such a limited margin would be considered inadequate or marginal in any analysis of local resection.

In our trial we learned the consequences of early (less than 5 days) application of BRT to a large healing wound,[6] as would be predicted by previous animal wound-healing studies.[14] The insistence that afterloading catheters not be loaded until the fifth or subsequent postoperative day has ameliorated the additive effect of wound-healing impairment by radiation.[5]

The current database has great potential for the use and correlation of alternative prognostic variables. An excellent example is the ability to examine the significance of biologic variables identified in the tumor with respect to outcome. We and others have demonstrated that tumors in which the expression of retinoblastoma-susceptibility gene product was decreased were more aggressive than tumors in which it was expressed by nearly all cells in patients with soft-tissue and bone sarcoma.[15,16] The diagnostic significance of cytogenetic differences in adult soft-tissue sarcoma also could be tested with such a data base.[17] We have suggested that these kinds of analyses have potential for further identification of high-risk groups within tumor grades.

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DISCUSSION

DR. EDWARD M. COPELAND, III (Gainesville, Florida): When I joined the faculty of the M. D. Anderson Hospital in 1972, convincing data from that institution had shown that adequate local excision of extremity soft tissue sarcomas followed by radiation therapy resulted in survival figures equal to those of amputation. Dr. Steven Rosenberg and his group several years later proved this observation in a randomized prospective trial, the data from which were presented before this Association.

Brachytherapy interests me because the time required for delivery is only 5 days rather than the 5 to 6 weeks required for adequate external beam radiation therapy. It is thus shorter and possibly less expensive. Please comment on the expense, because a prolongation of hospitalization to deliver brachytherapy may offset any cost savings by the shorter time that brachytherapy requires to deliver when compared with external beam radiation therapy.

What about preoperative radiation therapy? With the use of external beam radiation therapy, our experience has shown that large (more than 7 cm) soft-tissue sarcomas can be resected locally with minimal local recurrence. Even lesions abutting major neurovascular bundles can be locally resected by dissecting them from the vessels and nerve. Do you use preoperative radiation therapy? How do you handle these large lesions surrounding major neurovascular structures?

Do you use frozen section control of your surgical margins? It would appear that you do not, because patients in your series who had microscopic positive margins had no worse recurrence rate than did patients whose microscopic margins were free of disease. Personally I spend a lot of time in the operating room evaluating surgical margins. If you could convince me that this is unnecessary, a lot of time would be saved. Sarcomas do tend to grow up and down fascial planes; consequently a visibly free margin of 1 cm or more may contain sarcoma in the area of fascial covering over muscle groups and along neurovascular bundles. This observation is particularly important when treating liposarcomas. In fact, preoperative radiation therapy is a specific advantage for liposarcomas for this reason.

Lastly survival was not affected in your study. Local recurrence is reduced but survival was unaffected. The same appears to be true with the use of radiation therapy in the treatment of carcinoma of the rectum. Why have we not improved survival by reducing local recurrence?

Dr. Charles M. Balch (Houston, Texas): Dr. Brennan is to be congratulated for assembling a prospective database on sarcomas that will be a national resource for learning more about this uncommon but important disease. Dr. Brennan has performed a very well-designed controlled surgical trial that, it is hoped, will be the first of a series from Memorial Cancer Center. The Memorial Cancer Center surgeons, like many others, have moved away from more radical surgery, including amputation, to less radical surgery combined with radiation therapy to preserve the function of the limb.

It is important to account for not only those patients who are entered into a sarcoma trial such as this, but those who were eligible and did not enter. This is illustrated by a prior sarcoma study from Harvard Medical School that demonstrated that the patients who got into a prospective randomized trial in fact had a better prognosis and were not necessarily representative of those patients who refused to be entered into the trial (Antmas et al).

There are three goals of cancer surgery: cure, local disease, control, and staging. The goal of local disease control is undervalued by many nonsurgeons and even underemphasized by some surgeons when reporting their results. A local recurrence from the extremity sarcoma can result in additional surgery, painful debilitation, or even may lead to amputation, which is a terrible quality of life in a dying patient. In my view these are important results, demonstrating the value of multimodality therapy for improving local disease control, even if it did not demonstrate an improvement in overall survival.

Finally this is a study that illustrates the high risk of microscopic disease systemically that must have been present at the time of initial diagnosis and predominated the end results. It is clear that the feature advances in treating this disease will come with advances in controlling systemic component of the disease. To this end Dr. Raqhael Pollock and others at our institution have been conducting a prospective clinical trial using preoperative chemotherapy (using an Adriamycin combination), preoperative radiation therapy, followed by surgical excision—oftentimes using free composite grafts with microvascular anastomoses from unirradiated areas to minimize wound complications (Pezze et al, *Ann Surg* 211:476, 1990). Postoperative chemotherapy also is administered if the patient has a responsive tumor. This retrospective study was published last year; there was a 50% overall major response in these patients, and the 5-year survival rate was 85% for responders, but only 25% for nonresponders.

My questions are these, to Dr. Brennan: First what do you recommend as standard therapy for extremity sarcomas? Should all patients get brachytherapy, or is external beam irradiation equivalent? Second should radiation therapy be given preoperatively or postoperatively? And third what type of systemic therapy are you using today to try to improve survival rates?

Dr. John Daly (Philadelphia, Pennsylvania): Dr. Brennan and his colleagues have presented one of, if not *the* largest prospectively studied series of soft tissue tumors. Use of external beam radiotherapy and soft part resection when possible for these patients is the standard of care, but brachytherapy has not been.

I have several questions. The first is, in previous reports from Memorial and from other institutions, local regional recurrence has been associated with a poor outcome. This goes along with Dr. Copeland's question. Your follow-up median of 41 months and the survival curves that you showed strongly suggest that no difference will develop. Is this due to the effect of the Adriamycin that was given?

Secondly when local recurrence does occur in the nonirradiated group, how often could you obtain local control without the patient undergoing amputation?

Thirdly in low-grade tumors, should adjuvant radiotherapy be the standard of care?

Finally in 1987 you presented data on wound complications associated with brachytherapy, most of which occurred when the afterloading was done within the first 5 days after the operative procedure. Can you update us on the adverse results of this procedure that occurred in this entire series?

Dr. Paul Sugarbaker (Washington, DC): I think what Dr. Brennan tells us is that the rule of least margins still applies in patients with sarcoma. Surgery is beneficial in terms of survival even if large-volume, moderate-to-low-grade cancers are removed, even with a small margin. Sometimes even a patient with a positive margin with those large low-grade tumors benefits from surgery or surgery plus radiation therapy.

Unfortunately we all know that even the small tumors, where the least margin is the lungs or other sites that we do not detect, fail our most radical surgeries.

And so, Murray, for the most part, our work with extremity sarcoma has focused on quality of life benefits of our sarcoma treatments, and we have used induction, intra-arterial, and systemic chemotherapy to do basically the same as Dr. Brennan has shown. We improve local control, we spare extremities, and we have virtually no impact on survival, because we are not able, even with early chemotherapy, to control systemic disease.

So, Murray, are there quality of life benefits to your treatments?

Secondly can you tell us by any test, clinical, pathologic, biologic, whether one has a nonmetastasizing variant of a sarcoma, because we as surgeons can become very aggressive with these nonmetastasizing tumors. We need to be very conservative with tumors that are going to ultimately fail our treatments. Is there a test, Murray, that has come out of your database that tells us about the metastatic efficiency or inefficiency of these sarcomas?

Dr. John R. Benefield (Sacramento, California): Dr. Brennan has presented to us an extraordinary opportunity to examine the role of resection of pulmonary metastases in the management of these patients. The group of patients he has presented includes individuals we eventually see with one or more metastases. There is evidence in the literature to suggest that recurrent or repeated resection of these pulmonary metastases may—and I underline the word "may"—benefit these individuals. The available information, at the moment, is based primarily and almost exclusively on historic controls.

This wonderfully collected series of patients represent a data bank from which a prospective randomized trial could be done.

I am among the people who believe that the evidence regarding the benefits of resection of pulmonary metastases is sufficiently questionable to make it perfectly ethical and appropriate to conduct a prospective randomized trial, and I wonder, do Dr. Brennan and his group agree with this contention?

Dr. Murray F. Brennan (Closing discussion): I think it is important to emphasize that between 1968 and 1978 at our institution, 50% of people with extremity sarcomas underwent amputation. That number in 1990 is less than 5%.

The question of external beam *versus* brachytherapy is, it is clearly cost-effective? There are no data that brachytherapy is any less effective than external beam, so I would argue for its logistic role alone.

You asked about preoperative irradiation. I have no information on that and we are unlikely to ask that question, because it would require a logistic change to find somewhere for the patient to stay while we asked the question!

Frozen section? No, I do not use frozen section on the margins. There are two reasons for that. In the high-grade lesions, provided you stay outside of the tumor, I think the risk of significant positive margins is very low. I think it is important to emphasize that the present data demonstrate that microscopic margins, as defined by us, were not a factor in recurrence, and I will further analyze that.

In addition, in the low-grade lesions, it is almost impossible on frozen section for the truly fibrous tumors, the very low-grade fibrosarcomas, to define what a positive margin is.

You are absolutely right, Dr. Copeland, about survival, and the reason I stopped this trial in July of 1987 was to take out what I now believe can be defined as the very high-grade, large, deep lesions, because of their poor survival, and have entered them into a prospective trial examining the benefits (much as Dr. Balch emphasized), of preoperative chemotherapy. We have not published any of that yet, but at present we have not shown a significant benefit in survival for most lesions. It is true that some of them decrease in size.

We have not been as successful as Dr. Frederick Eilber and Dr. Gerald Rosen, but perhaps that is because we have used lesser doses of ifosfamide. I think their work is very interesting.

Dr. Balch, I thank you for your remarks. I believe I answered the question about amputation. You were kind enough to suggest that you hoped this was the first of many prospective randomized trials from Memorial. I would like to reassure you that that was so, but randomized trials at our institution are still not easy! The concept that we all learn by osmosis is well entrenched, as you appreciate.

As for the different outcome in nonrandomized trials, we very carefully followed the patients who were not randomized, and I gave you that data. I think it is interesting, as in other randomized trials, that the nonrandomized group does better. You are absolutely correct. I would like to believe that that is a product of clinical judgment and we should be reassured.

Regarding the systemic disease question, I answered that in part. We do have a prospective trial examining the high-risk group. That is not a randomized trial. I view it much more as the questions we would ask in pancreatic cancer. If you have anything that works—that is, if you threw a brick out the window and it went up—you do not need a randomized trial. At present we have not shown a survival benefit in that trial.

You asked for the recommendations. At present I do think that patients with high-grade lesions should receive radiation therapy after their resection, although for the small, less than 5-cm, superficial lesions, that could be argued. For the low-grade lesions, it is very interesting. The numbers are not large, but we have not shown a benefit with adjuvant radiation therapy in the low-grade group, and so I have continued a randomized trial looking at the benefit of radiation to low-grade lesions. That is a hard trial to do once you have suggested that it has a benefit in any sarcoma tumor.

Dr. Daly, thank you for the questions. There was no effect of the Adriamycin that we could identify. I cannot give you the exact numbers for the people we salvaged after local recurrence, but I will obtain them for you. We certainly were able to salvage some people in both arms of the trial, with a recurrence locally by a further operation. It was the reason that I described in the data the first recurrence only, because I thought that was the meaningful question to answer, rather than long-term disease, free from local recurrence.

I answered the question about low-grade. I think it is highly debatable whether they should receive adjuvant radiation, and that is an opportunity for study.

As for wound complications, because we have moved to brachytherapy no sooner than the fifth or sixth day, the complication rate in these big operations is the same, whether they receive radiation therapy or not. Parenthetically that is an example of something we learned in the laboratory 10 years ago by a study designed to answer when radiation could be used after wounding.

Dr. Sugarbaker, I appreciate your comments. Again the rule of least margins, as you describe it, I think is appropriate. I am very sensitive to the high quality of your earlier studies looking at quality of life between amputation and resection, and I think that illustrates it.

The question of induction chemotherapy I have answered. We have not as yet shown an impact on survival, but I am hopeful that we have just picked the wrong regimen and that Dr. Eilber will be proved to be correct.

The follow-up slide would answer the question that you asked about prediction.

As I wait I would answer Dr. Benfield. He asked a very important question. At present, all data suggest that patients with pulmonary metastases who undergo complete resection for metastatic soft tissue sarcoma have a 5-year survival rate of 20% to 38%. Those are the data as published. He is absolutely correct to have some concern about that information, because we do not know what the denominator is. I am unwilling to ask the randomized trial yet, Dr. Benfield, but what I should be able to provide for you is a group of patients, over 700 now, entered with primary tumors, some of whom will go on to have pulmonary metastases, and will or will not become resected. We would then have a number that would tell us whether the true number is 20% or indeed if this is an extraordinarily highly selected subset, and a randomized trial would not be justified.

The second reason that a randomized trial probably will not be justified is that there is no question that there are *some* patients salvaged by pulmonary resection of soft-tissue sarcoma, and to my knowledge there has never been in adult non-rhabdomyosarcoma a cure by any other form of therapy, but I should be able to provide that information for you.

I am sorry. I did try to anticipate some of the questions with some other slides, but they are unavailable, so I will answer Dr. Sugarbaker's question and close. It is very clear at present that the most important thing that you can do for the patients is to clearly categorize the tumor with which they present. It is absolutely inappropriate to embark on a program of management without clear knowledge of the histopathology, the subtype of that histopathology, the grade, and then the things I mentioned: site, size, and vascular involvement. With those factors alone, we can clearly predict with a high accuracy the likelihood of subsequent metastases.

Perhaps most exciting, however, is the opportunity we have had now to review some of the molecular biologic determinants. Our most recent study looking at the expression of the retinoblastoma gene product was done by Dr. William Cance, formerly working with Dr. Wells before he came to us, and now working with Dr. Sheldon. This work has shown very clearly that if that gene product is deleted, or is absent, then the 2-year survival is 28%. In contradistinction if that tumor suppressor gene product is expressed homogeneously on all of the tumors, then the 2-year survival is 71%.

Now I should emphasize that we are making big decisions on 13 patients and 13 patients. But this is the kind of information that I think this data base will help to provide in answering the question that Dr. Sugarbaker asked, that the expression of some of these gene products will be able to describe for us survival that is very poor *versus* survival that is very good in high-grade, large sarcomas, already taking out the predictive factors that we know to be of importance.

Transplantation of Islet Allografts and Xenografts in Totally Pancreatectomized Diabetic Dogs Using the Hybrid Artificial Pancreas

ANTHONY P. MONACO, M.D.,* TAKASHI MAKI, M.D., PH.D.,* HIROKI OZATO, M.D.,* MAURO CARRETTA, M.D.,*
SUSAN J. SULLIVAN, PH.D.,† KERMIT M. BORLAND, PH.D.,† MICHELE D. MAHONEY, M.S.,† WILLIAM L. CHICK, M.D.,†
THOMAS E. MULLER, PH.D.,‡ JACQUELINE WOLFRUM, PH.D.,‡ and BARRY SOLOMON, PH.D.‡

Previously the authors reported on a Hybrid Artificial Pancreas device that maintained patent vascular anastomoses in normal dogs and, when seeded with allogeneic canine islets, maintained normal fasting blood sugars (FBS) in diabetic pancreatectomized dogs. Eventual failure of these devices was believed to be related to loss of islet viability and/or insufficient islet mass. The current study evaluates the effect of increased islet mass produced by implantation of two islet-seeded devices in pancreatectomized dogs and compares the results with those from dogs that received a single device. Twelve of fifteen dogs receiving single devices showed initial function as determined by elimination or reduction of exogenous insulin requirement; four showed initial function and seven showed extended function (100 to 284 days). Excessive weight loss (more than 20%), despite normal FBS and insulin dependence, required that four animals in this latter group be killed. Devices seeded with xenogeneic islets have met with limited success. One dog that received two bovine islet-seeded devices achieved function for more than 100 days; the remaining bovine-seeded devices (n = 8) functioned for only 3 to 16 days. Porcine islet-seeded devices functioned for 14 and 80 days. Metabolic effects of the devices were assessed by intravenous glucose tolerance tests (IVGTT). Recipients of two devices seeded with allogeneic islets demonstrated improved IVGTT results when compared to those from pancreatectomized dogs and recipients of single devices but were abnormal when compared to intact animals. Histologic examination of device and autopsy material from all failed experiments was performed and showed no mononuclear cell infiltration of the islet chamber or vascular graft material, only a few incidence of device thrombosis, and varying degrees of islet viability as judged by morphologic and immunohistochemical evaluation. The authors believe they have demonstrated progress toward the development and clinical applicability of the Hybrid Artificial Pancreas.

CLINICAL PROGRESS IN both renal and extrarenal whole-organ transplantation in the last decade has been phenomenal. Replacement of vascularized whole organs is common and generally considered the most effective treatment for a number of morbid and fatal diseases. This success is due, in part, to understanding the immunologic mechanisms of allograft rejection and to the prevention and treatment of rejection with increasingly effective immunosuppressive agents. Unfortunately chronic maintenance immunosuppression is associated with a number of complications: opportunistic infection, increased incidence of spontaneous neoplasms, failure to control rejection, metabolic alterations secondary to drugs, and direct drug toxicities. Nevertheless the benefits of successful whole-organ transplantation far outweigh the risks and complications of chronic immunosuppression in most situations.

Despite efforts to control blood glucose levels with diet, exercise, and exogenous insulin, patients with insulin-dependent diabetes mellitus continue to sustain secondary vascular, neurologic, and other complications. Although still controversial, the current weight of evidence suggests that this is due to failure to achieve normal physiologic control of blood sugar levels by these methods. Because of this whole-pancreas transplantation has been undertaken with increased frequency and improved success.[1] Excellent blood glucose control and insulin independence has been achieved after pancreas transplantation. Unfortunately, to date, reversal or stabilization of diabetic complications has been equivocal or unsubstantiated,[2-5] presumably because most pancreas transplants have been performed in patients with already-established late complications. Although patient and graft survival rates have improved steadily, whole-pancreas allotransplantation has

From the Division of Organ Transplantation, Department of Surgery, New England Deaconess Hospital and Harvard Medical School, Boston, Biohybrid Technologies Inc.,† Shrewsbury, and W. R. Grace Company, Biomedical Research Division, Lexington, Massachusetts‡*

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Address reprint requests to A. P. Monaco, M.D., New England Deaconess Hospital, Division of Organ Transplantation, 185 Pilgrim Rd., Boston, MA 02215.

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the disadvantages of the risks of major surgery, the necessity for chronic immunosuppressive therapy, and the limited applicability because of shortage of donor organs. Although it is becoming apparent that pancreas transplantation should be done early to prevent development of complications, there is a natural reluctance to risk a major surgical transplant under significant immunosuppression very early in the course of diabetes when the disease is well controlled with insulin, the patient is free of complications, and when it is not known that such complications will occur in a given patient.

Islet transplantation has been studied extensively as an alternative to whole-pancreas transplantation. Islet transplantation has a number of practical and theoretical advantages. These include major surgery (and its potential complications) are avoided; multiple donors can be used as islet sources[6,7]; islets can be stored easily and frozen until used[8,9]; xenograft islets theoretically could be used[10]; and islets can be modified (immunomodulation) by culturing and *in vitro* other techniques to decrease immunogenecity.[11] The disadvantages of islet transplantation are that large numbers of human islet allografts are not available, large-scale isolation procedures are not well developed,[12] and islets are still of sufficient immunogenecity to require large amounts of immunosuppression to prevent rejection.[13] Nevertheless progress in clinical islet allografting was recently made.[11,14,15]

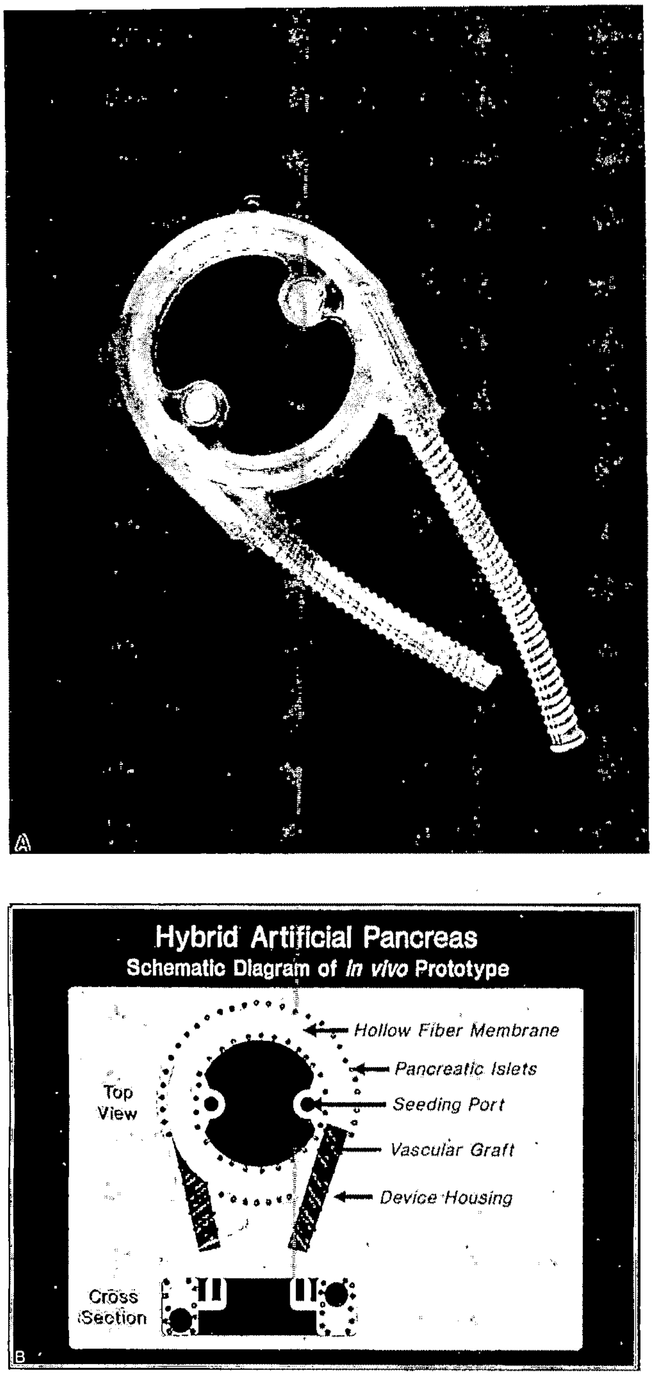
Because cellular transplants, such as islets, do not require large-vessel surgical anastomoses, they have the additional theoretical advantage of transplantation under conditions that produce a physical barrier between transplanted cells and the recipient's immune system, thus circumventing rejection and obviating the requirement of immunosuppression. In most barrier isolation systems, islets are separated from the host by some type of semipermeable membrane that allows molecules of defined size to pass through. Small molecules, including glucose, insulin, and other nutrients, are exchanged across these membranes, while effector lymphocytes, immunoglobulins, and other transplant rejection effector mechanisms are excluded. Two general kinds of immunoexclusion methods have been studied. In microencapsulation methods, single islets or groups of islets are enclosed or encapsulated by semipermeable membranes of structured organic components (such as alginate).[16,17] Microcapsules can be implanted into the peritoneal cavity or some other anatomic location. Nutrients pass across the membranes to nourish islets from the surrounding extracellular fluids and insulin secreted in response to glucose levels passes across into the extracellular fluid and is eventually absorbed into the circulation. These devices, in which the membrane is in direct contact with extracellular fluids, have been limited by fibrosis around the membrane, which eventually prevents adequate solute exchange. Progress in understanding and preventing fibrosis has been made.[18] A variation of this diffusion concept involves creation of larger diffusion chambers composed of semipermeable membranes holding a larger number of islets. In the second kind of immunoexclusion device, the semipermeable membrane is formed into a hollow tube, which is contained in a housing that is connected to a circulatory system of the host as an arteriovenous shunt. Islets are placed inside the device but outside the hollow tube membrane. Glucose, nutrients, and oxygen cross the membrane directly from the blood. Insulin produced by the islets diffuses directly into the blood stream. Rejection of islets is prevented because immune lymphocytes and immunoglobulins are excluded by the semipermeable membrane. In this type of exclusion device, the membrane is in direct contact only with blood and no other body tissues or fluids. Fibrosis of the membrane thus may be less likely to occur. Also, such macroencapsulation, intravascular exclusion devices depend on the nonthrombogenecity of the semipermeable membranes on contact with the blood stream.

We have previously reported initial studies using the Hybrid Artificial Pancreas device, which is suitable for implantation into larger animals.[19] In these initial studies we showed that the device could be implanted unseeded (without containing islets) into normal dogs and remain patent for almost 1 year and be well tolerated by the recipient animals. We also reported initial studies in which single devices containing canine allograft islets could maintain completely pancreatectomized dogs with normal fasting morning blood glucose levels with reduced or absent exogenous insulin requirements for varying periods of time without the need for immunosuppression. Recently we also reported the insulin output and response kinetics of seeded devices perfused *in vitro* with variable glucose concentrations.[20] Furthermore, because the current device configuration limits the amount of insulin output per device, efforts have been made to increase the insulin available *in vivo* for implanted diabetic recipients by implantation of two devices.[20] In this paper we report in detail the control of diabetes in pancreatectomized dogs by implantation of two devices seeded with canine allografts. We also report the results of initial experiments in which devices seeded with bovine and porcine islets were similarly transplanted to pancreatectomized diabetic dogs.

Materials and Methods

Hybrid Artificial Pancreas Device

The design of the Hybrid Artificial Pancreas used a modification of the technology that developed for a hollow fiber cell culture device. The prototype device consists of a single-coiled, hollow fiber membrane contained within a disk-shaped, acrylic housing (Fig. 1) that provides a



FIGS. 1A and B. (A) Photograph of a hybrid artificial pancreas device and (B) schematic representation of the device.

compartment for the islet cells. The specially prepared 5- to 6-mm internal diameter (ID) membrane was fabricated from an acrylic copolymer material using a modified solution spinning technique previously described.[21] The resulting ultrafiltration membrane has a nominal molecular weight cut-off of approximately 50,000 daltons (50 kd). An annular cavity surrounds the tubular membrane and defines the islet-containing chamber within the device. Access to this cavity can be achieved through two syringe ports capped with medical grade silicone. The Hybrid Artificial Pancreas device measured 9 cm in diameter, is 2 cm high, and weighs 50 g. The annular-shaped acrylic housing contained 30 to 35 cm of coiled, tubular membrane (ID, 5 to 6 mm) with a wall thickness of 120 to 140 nm. As configured the device provided approximately 60 cm^2 of membrane surface area and compartment volume around the membrane of 5 to 6 mL (Fig. 2). For these devices to be used for *in vivo* implantation, the venous and arterial ends of the hollow fiber membrane were connected to polytetrafluoroethylene (PTFE) vascular grafts with a matched inner diameter and an external coil wrap (IMPRA, Tempe, AZ). They were sealed into the housing using a medical grade epoxy. For those devices to be perfused *in vitro* (see below), the ends of the hollow fiber membrane were sealed directly within the acrylic tubing flanges. Both *in vivo* and *in vitro* devices were sterilized using ethylene oxide gas and aerated before use.

Islet Isolation and Device Seeding

Pancreata were surgically removed from dogs under general anesthesia with zero to minimal warm ischemia time. Islets were isolated using a collagenase digestion and discontinuous Ficoll density gradient method as described by Warnock and Rajotte.[22] Islets isolated from two to

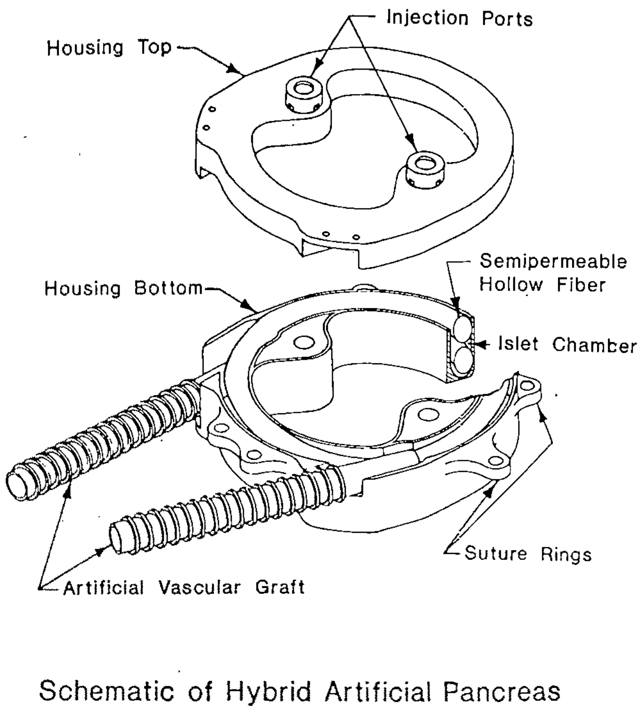

Injection Ports
Housing Top
Semipermeable Hollow Fiber
Housing Bottom
Islet Chamber
Suture Rings
Artificial Vascular Graft
Schematic of Hybrid Artificial Pancreas

FIG. 2. Schematic diagram of the hybrid artificial pancreas device showing the relationships of the islets to membrane and blood stream.

three dogs were pooled before use. Islet purity exceeded 95% as determined by dithizone staining, amylase content, and histologic analysis. On the day of device implantation, isolated islets that had been in culture for 24 to 72 hours (see below) were collected by centrifugation. The islet pellet was resuspended in M199/EBB medium (Sigma Chemical Co., St. Louis, MO) supplemented with 5% fetal bovine serum, 40 IU/mL penicillin, 0.5 mol/L (molar) HEPES, and 2 mmol/L (millimolar) glutamine, and 1% agar. An aliquot of islets was taken for control culture dishes. The remaining islets were divided according to the number of devices being seeded. The islet suspension was injected into the device through the seeding port. The *in vivo* devices were transported on ice to the surgical site for implantation. Perfusion of *in vitro* devices was begun at the time *in vivo* devices were surgically implanted.

For those experiments in which xenograft islets were used, bovine pancreata were obtained from local abattoirs after animals were slaughtered in standard fashion. Pancreata were then removed with an extended warm ischemia time of 15 to 30 minutes. Porcine pancreatic glands were obtained by surgical removal using methods similar to those described for canine glands. Thereafter the islet isolation, purification, and device seeding procedures were essentially the same as with canine islets.

Culture of In Vitro *Control Devices*

For each experiment a corresponding *in vitro* control device was seeded at the same time and under the same conditions as the *in vivo* device and was placed in perfusion culture at 37°C in a humidified atmosphere of 5% CO_2 in air, as recently reported.[20] The *in vitro* devices were perfused with M199/EBSS medium containing 200 mg/dL glucose. The culture medium circulated through the device at a flow rate of 100 mL/minute using a peristaltic pump. The medium was changed three times per week. A sample was taken at each change for determination of immunoreactive insulin concentrations and stored at −20°C until assayed.

Measurement of Insulin Release

Determination of insulin levels in the *in vitro* perfusion studies was done using a previously reported radioimmunoassy protocol.[20] Free antigen was separated from the antigen–antibody complex by precipitation with IgGsorb (*Staphylococcus aureus* protein A, The Enzyme Center, Boston, MA). The limit of assay sensitivity was 25 U (1 ng)/mL.

Animals

Female mongrel dogs weighing 15 to 20 kg were purchased from Biomedical Association, Inc., Friedensbury, PA. In some experiments female beagles weighing 10 to 15 kg also were used. All dogs were examined by a veterinarian to ensure their health and were housed in compliance with United States Department of Agriculture (Animal Welfare Act) Regulations Part III at the Animal Resources Center facility of Harvard Medical School. In compliance with humane care considerations of the Animal Care Committee of Harvard Medical School, no animal could undergo more than three surgical procedures and animals that lost 20% or more of preoperative body weight were killed.

Surgical Procedures

Anesthesia. Baseline body weight, blood chemistry, and hematologic profiles were obtained before surgery. Animals were fasted overnight before surgery but had free access to water. Atropine (0.01 mg/kg) and, if required, acepromazine maleate (0.5 mg/kg), was administered intramuscularly 30 to 45 minutes before surgery. Anesthesia was induced with intravenous 4% Bio-tal (thiamylal sodium 0.5 mL/kg) injection and maintained with 1% to 2% halothane in 100% oxygen administered *via* an endotracheal tube. Lactated Ringer's solution (50 mL/kg) was given intravenously during surgery.

Pancreatectomy. Total pancreatectomy (more than 95%) was performed through a midline incision. The tail of the pancreas was dissected from the splenic vein, portal vein, and surrounding mesentery. The duodenal portion of the pancreas was separated from the duodenal wall. Care was taken not to damage the duodenal artery and vein that are situated parallel to the duodenal arch. Small vessels were either cautery coagulated or ligated and divided. The pancreatic duct was identified approximately 5 cm below the gastroduodenal junction and dissected in preparation for division. The head of the pancreas was dissected from the surrounding vessels and mesentery and the gland removed. On completion of pancreatectomy, the mesenteric defect was closed with 4-0 chromic catgut.

Device Implantation. Device implantation was performed 2 to 3 weeks after pancreatectomy, during which time diabetes was treated by daily exogenous insulin. Laporatomy was performed through a vertical midline incision and the iliac vessels were exposed. The arterial limb of the device was anastomosed end to side to the left common iliac artery with continuous 6-0 Prolene and the venous limb was similarly anastomosed end to side to the right common iliac vein. During implantation no heparin was used except for flushing of the vessels before anastomoses. On completion of the anastomoses, the intestines was covered with the omentum and the device was placed between the omentum and the anterior abdominal wall. The device was fixed to the abdominal wall with interrupted 2-0 Ethilon sutures (Ethicon, Somerville, NJ). The abdominal wall muscles and the skin wound were then closed with continuous 2-0 and 3-0 Ethilon, respectively. For those dogs in which two devices were implanted, the same techniques were used except that the arterial and venous limbs of the second device were anastomosed to the right common iliac artery and left common iliac vein, respectively.

Postoperative Care

Immediately after surgery, dogs were covered with blankets until they were fully awake. Acepromazine maleate and/or pentazocine lactate (Talwin; Winthrop Laboratories, New York, NY) was administered for pain relief if necessary. Water was given *ad libitum* but food was withheld until the second postoperative day. Amoxicillin (50 mg) was given for 3 days starting from the day of surgery. After operation multivitamin tablets (Vita-Min Palatabs, ESSAR Corp., Fort Dodge, IA; 6 tablets a day) and, in the case of pancreatectomized dogs, pancreatic enzyme tablets (Viokase-E, A.H. Robins Co., Richmond, VA; 2 tablets a day or equivalent as Viokase powder) were administered every day mixed with food. Aspirin (75 mg) was administered every day to animals in which devices were implanted. Vascular patency of the device was determined daily by the presence of a bruit(s) over the device(s). In the absence of a bruit on 2 consecutive days, the seeded device(s) was removed under general anesthesia using sterile procedures. Dogs from whom seeded devices were removed were subsequently killed when blood glucose concentrations returned to preimplantation levels.

Metabolic Studies and Postoperative Insulin Requirements

In pancreatectomized dogs with or without seeded devices, fasting morning blood glucose levels were determined using Chemstrip tapes as well as a Beckman glucose analyzer (Beckman Instruments, Brea, CA). Glucose values determined by chemstrip tapes correlated well with those obtained using the glucose analyzer (Spearman correlation, $R = 0.98$ with $n = 100$). Subcutaneous porcine lente insulin was administered once a day to maintain blood glucose levels between 150 and 250 mg/dL. Animals were weighed two to three times a week after pancreatectomy and seeded device implantation. Because of the tendency for hypoglycemia to occur in pancreatectomized dogs with excess exogenous insulin, no attempt was made to keep blood glucose levels in the 100 to 150 mg/dL range.

Intravenous glucose tolerance tests were performed between pancreatectomy and device implantation and periodically following device implantation on all dogs with functioning devices. A blood sample was taken for a baseline glucose level and then 50% dextrose (0.5 mg/kg) was infused intravenously. In pancreatectomized dogs, 0.25 mg/kg of dextrose was used. Further blood samples for glucose determination were taken 5, 10, 20, 30, 40, 50, 60, 90, and 120 minutes after injection. Postprandial glucose tolerance was determined by measuring blood glucose concentrations serially before and after standard meal feeding.

Histologic Studies

A complete gross and microscopic examination was performed on each removed device. All specimens were fixed in 10% buffered formalin and processed by standard techniques. Tissue sections were stained with hematoxylin and eosin. Appropriate sections of vascular grafts were studied to document anastomosis-related fibrous hyperplasia. Viability of islets was estimated by examining the H&E-stained islets under ×160 magnification. Islets were examined for the presence of central necrosis, generalized loss of nuclei or pyknotic nuclei, and reduction of cell size due to necrosis. An estimate of the percentage of viable islets was made using the extent of the presence of these changes in the individual islets. Also sections containing islets were stained with an immunoperoidase method to demonstrate stored insulin within the beta cells.[20] Complete autopsies were performed on all dogs in these experiments, except for those animals that received unseeded devices and those whose seeded devices are still functioning.

Results

Long-term Follow-up of Implanted (Unseeded) Devices in Normal Dogs

In our initial report,[19] two series of normal dogs were implanted with single devices that were not seeded with islets (unseeded devices). Table 1 presents updated results on these dogs, which have now been followed for an additional 8 months. In the first series, the arterial limb of the device was anastomosed end to end to the common iliac artery and the venous limb end to side to the common iliac vein. One dog maintained device patency for 560 days and another for 663 days. In the second series of dogs, the anastomoses were performed end to side (except

TABLE 1. *Summary of Unseeded Device Implantation*

Group	n	Device Patency (days after implantation)
I	12	4, 8, 18, 16, 26, 28, 50, 63, 170, 270, 560, >663
II	8	267, >514, >521, >530, >530, >537, >537, >544

Group I: End-to-end arterial anastomosis; no aspirin.
Group II: End-to-side arterial anastomosis; low-dose aspirin.

for two dogs) and all dogs received daily aspirin and perioperative antibiotics. Currently seven of eight of these normal dogs have patent devices implanted for more than 500 days confirmed by audible bruits and doppler ultrasonography. In both series of dogs, no significant weight loss or health abnormalities have been observed through the present time.

Metabolic Effects of Pancreatectomy

Since our initial report[19] in which 26 dogs had been completely pancreatectomized in preparation for device implantation, an additional 41 total pancreatectomies have been performed. All but one of these 60 dogs became hyperglycemic (more than 300 mg/dL) within 24 hours. The single dog that survived and was normoglycemic had a normal IVGTT after surgery; presumably fasting normoglycemia was maintained by residual pancreatic tissue. The failure to induce diabetes by our method of total pancreatectomy was less than 1.5%. Insulin dose required to maintain blood glucose concentrations in the 150 to 250 mg/dL range during the period between pancreatectomy and placement of seeded devices averaged from 18 to 32 units/day. As mentioned above blood glucose values were maintained at relatively high levels (approximately 200 mg/dL) because of the difficulty in maintaining constant normal glucose levels (100 to 125 mg/dL) in pancreatectomized dogs. In addition, despite the use of exogenous insulin, pancreatic enzymes and multiple vitamins, many of the dogs lost approximately 10% of their prepancreatectomy body weight in the 2 to 4 weeks before placement of seeded devices.

Effect of Single Devices Seeded with Canine Islet Allografts

Table 2 lists the postoperative insulin requirements of 15 pancreatectomized dogs implanted with single devices seeded with varying numbers (12,000 to 22,000 islets/kg) of canine islet allografts (13 previously implanted and reported,[19] and an additional two dogs subsequently transplanted). The average daily insulin requirement for each week after operation is listed for the first 14 weeks. Devices were defined as nonfunctioning on the postoperative day that exogenous insulin requirements reached 50% or more of the preoperative exogenous insulin dose. Devices in three dogs (PS11, PS12, PS16) failed to alter significantly the fasting blood glucose levels and were considered to be failed (F) despite successful initial surgery. The remaining 12 dogs had varying degrees of reduced insulin requirements for 14 to 280 days. Dogs PS19 and PS20 showed only minimal function for 1 to 2 weeks, failure being attributed to loss of islet function (device patent) in dog PS19 and infection in dog PS20. Five dogs demonstrated function for 42 to 76 days (PS7, PS8, PS18, PS21, PS25) and five dogs had function for 103 to 280 days (PS9, PS10, PS15, PS17, PS27). Note should be made that four dogs (PS7, PS10, PS15, PS27) had no insulin requirements for long periods during the first 14 weeks after transplantation. Stable glucose levels of 100 to 200 g/dL were achieved in some dogs immediately after implantation and in others only after 2 or 3 weeks. Of the three dogs with no significant function, failure was due to collapse of membrane and vascular graft (PS11), thrombosis (infection) (PS12), and islet failure (PS16). Of the 12 dogs that showed any significant post-transplant function, loss of device function

TABLE 2. *Insulin Requirement After Single Seeded Allograft Devide Implantation*

	Mean Insulin Requirement (unit/day)																
		Postimplantation Week															
	Pre-Device −1 Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Function (days)	Cause of Failure
PS7	16.9	0	0	0	0	0	0	0	0.6	0	0	1.4				76	Clotting
PS8	19.1	2.6	0	0	3.0	8.0	10.0									36	Islet
PS9	24.9	6.0	8.9	2.0	3.7	3.1	4.7	5.4	14.0	16.0	10.0	3.1	8.0	8.0	8.5	141	Islet
PS10	10.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	280	Unk
PS11	34.3	14.0	29.0	34.0												F	Device
PS12	22.5	9.4	18.6	22.0												F	Clotting
PS15	18.3	7.4	7.0	4.0	0	0	1.4	4.9	4.3	4.0	1.7	0	0	0	0	103	Clotting
PS16	14.0	9.4	20.3	22.0	17.3											F	Islet
PS17	28.3	5.7	9.4	10.0	10.6	12.9	14.0	12.3	10.0	17.7	18.0	16.6	18.0	16.6		64	Islet
PS18	24.3	9.1	12.6	10.9	10.3	14.0	19.0									31	Islet
PS19	18.0	2.9	12.3													9	Device
PS20	19.7	7.4	13.1	13.3												4	Infect
PS21	19.4	10.6	19.4	10.6	4.3	5.7	15.6	16.3	18.3	22.9	21.5					3	Islet
PS25	23.1	4.3	14.6	17.1	16.3	12.6	11.1									8	Islet
PS27	18.6	0	0	0	6.9	0	0	0	0	6.0	6.0	6.0	0	0	0	139	Islet

F, no function.

ISLET, failure of glucose control attributed to loss of islet function; anastomosis patent through postoperative course.

was attributed to late thrombosis in two (PS7 and PS15), membrane collapse (PS19), infection (PS20), and loss of islet function (viability) with patent devices (PS8, PS9, PS17, PS18, PS21, PS25, PS27). Dog PS10 functioned for 280 days and was found dead in his cage; autopsy failed to identify an obvious cause of death, although the animal had sustained significant (15%) weight loss.

We have reported previously that in animals in which single-device implantation was successful (as measured by reduced insulin requirements), the function of the device may be considered partial or complete. Figure 3 is taken from our previous publication[19] and illustrates the representative postimplantation course of animals with partial function. In dog PS15 up to 24 units/day of insulin were required before implantation to maintain fasting blood glucose levels at approximately 300 mg/dL. Blood glucose concentrations gradually returned to normal (100 mg/dL) after device implantation followed by relatively stable levels at approximately 200 mg/dL. Accordingly minimal doses of insulin (0 to 6 units/day) were required during this period. The dog was killed on day 103 after implantation because of loss of bruit associated with an abrupt increase in blood glucose levels. Histologic examination revealed thrombosis at the venous anastomosis

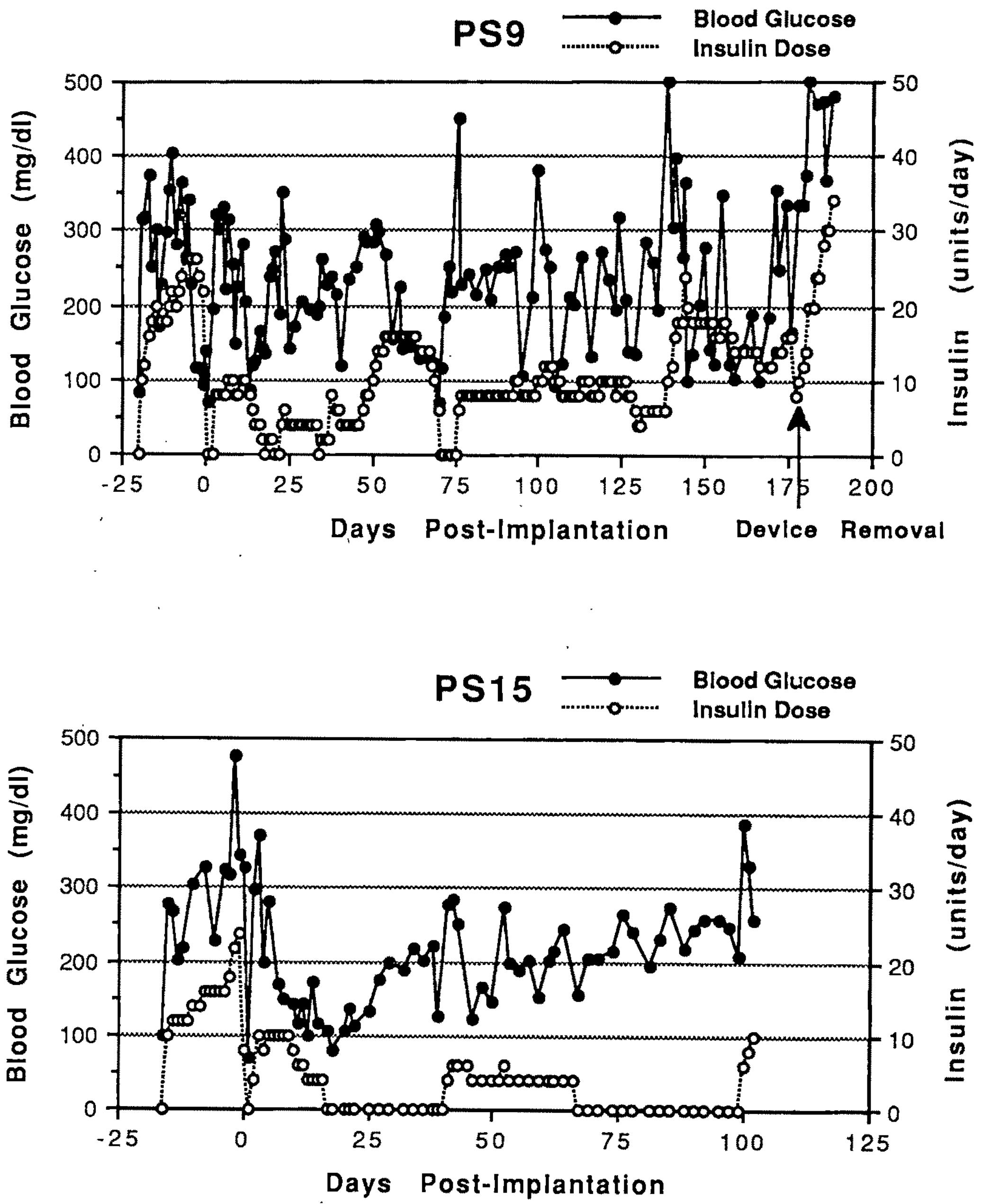


FIG. 3. Fasting blood glucose levels and exogenous insulin requirements in two dogs following implantation of single devices (allograft seeded) that show partial function. Dogs (PS15 and PS9) were pancreatectomized on days 16 and 20, respectively, relative to device implantation on day 0. Dog PS15 was killed on day 103 after transplantation because of loss of bruit. Dog PS9 had the device removed on day 177 after transplantation because of loss of function (day 175).

secondary to stenosis resulting from fibrous hyperplasia. Dog PS9 required much less insulin during the postimplantation period than during the 3 weeks before device implantation (up to 26 units/day); nevertheless it was extremely difficult to stabilize blood glucose levels in this severely diabetic dog. With removal of the device, however, the insulin dose increased from 16 to more than 32 units/day, confirming the finding that the implanted device had maintained some function, although it was not adequate to restore normoglycemia. Histologic examination of the removed device showed no cellular infiltration anywhere in or around the islets. Approximately 17% of islets were viable and contained insulin as evidenced by immunoperoxidase staining.[19]

Figure 4 taken from our previous report[19] illustrates the postimplantation courses of two dogs (PS10 and PS7) that showed long-term full function of the device. In dog PS10 blood glucose levels were as high as 350 mg/dL and up to 18 units/day of insulin was required before implantation. After implantation fasting blood glucose levels immediately returned to normal (100 to 125 mg/dL) and no insulin was required after the third day after implantation. However the fasting blood glucose levels slowly began to increase from 100 to 140 mg/dL to 150 to 250

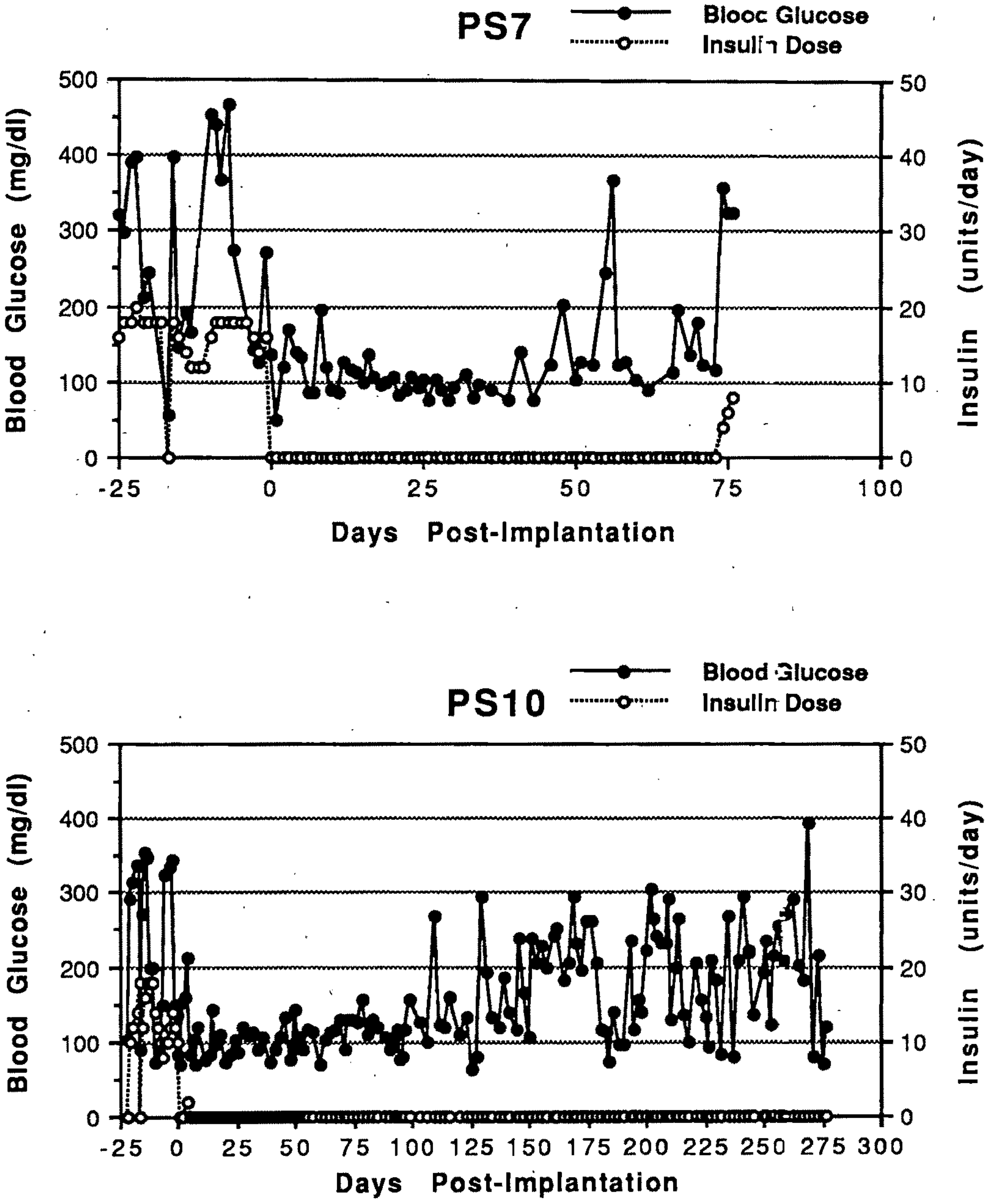


FIG. 4. Fasting blood glucose levels and exogenous insulin requirements in two dogs after single device implantation (allograft seeded) that show no further requirements for exogenous insulin. Dogs PS10 and PS7 were pancreatectomized on days 24 and -30, respectively, relative to device implantation on day 0. Dog PS7 had the device removed on day 76 after implantation because of functional deterioration subsequently found to be secondary to vascular thrombosis.

mg/dL after 125 days after implantation. The dog continued to do quite well with blood glucose values in the 150 to 250 mg/dL range without exogenous insulin for the next 150 days. This dog died (cause unknown) on postoperative day 280 off insulin with a patent functioning device. Dog PS7 required 17 units of insulin per day after pancreatectomy with poor fasting blood glucose control. After transplantation there was excellent glucose control (100 to 125 mg/dL) with no insulin requirements. The device was removed from dog PS57 on day 76 because of functional deterioration (thrombosis).

Effect of Double Devices Seeded with Canine Islet Allografts

In recent studies[20] we demonstrated that the output of the seeded devices as currently configured is limited by the maximum number of islets that can be placed in the device volume. Pending redesign of the hybrid pancreas device, experiments were done to increase the output of insulin available to pancreatectomized dogs by implanting two seeded devices (Table 3). All 13 dogs implanted with double devices showed some degree of device function. Four dogs had function for 100 to 284 days (PS22, PS23, PS26, PS36) and three dogs are still functioning more than 78 days (PS56), more than 162 days (PS50), and more than 125 days (IP-1) after transplantation. The remaining six dogs had function from 26 to 72 days. One dog (PS23) died of postoperative small bowel obstruction with a functioning device with patent anastomosis. Four dogs (PS28, PS30, PS36, PS49) were killed with functioning devices because of excessive weight loss despite the fact that they were off exogenous insulin at the time they were killed. All four dogs were found to have patent anastomosis (dog PS28 had one device anastomosis occluded). By our laboratory rules, if any animal loses up to 20% body weight the animal is killed on the basis of humane care. One dog (PS48) lost function on day 72 from clinically occluded devices (loss of bruit) confirmed at autopsy. Four dogs (PS22, PS24, PS26, PS35) lost function with clinically patent devices (audible bruits). Both devices at autopsy were patent in dogs PS22, PS24, and PS26 and the cause of failure of these devices was attributed to loss of islet function. Dog 35 had one (left) device patent and cause of failure was also attributed to loss of islet function. Thus implantation of two devices was more effective in controlling diabetes in totally pancreatectomized dogs. All dogs showed some function and 10 of 13 dogs were completely free of exogenous insulin for a significant period of time.

Figure 5 illustrates the first 200 days after transplantation course of dog PS22, which required approximately 18 units of insulin/day before double-device implantation. Note that for the first 100 to 150 days the fasting morning glucose levels were well controlled and were stabilized in the range of 100 mg/dL. During the next 50 to 75 days, the dog required no insulin, but the initial low glucose levels gradually increased. After 200 days the animal intermittently required 4 to 6 units of insulin to maintain the blood glucose level under 200 mg/dL. By day 284 this dog required up to 10 units of regular insulin per day and the devices were declared nonfunctioning. The devices were recovered and vascular anastomoses were patent.

TABLE 3. *Insulin Requirement After Double Seeded Allograft Device Implantation*

	Mean Insulin Requirement (unit/day)																
		Postimplantation Week															
	Pre-Device −1 Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Function (days)	Cause of Failure
PS22	18.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	263	Islet
PS23	20.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	209	Ileus
PS24	26.6	12.6	16.3	12.3	7.4	4.3	0	12.3	7.7	8.6	14.0	17.4	20.0	22.5		64	Islet
PS26	27.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	169	Islet
PS28	22.9	5.7	2.9	3.1	0	0	0	0								51	BW↓
PS30	16.3	0	0	0	0											28	BW↓
PS35	29.7	12.9	16.3	10.0	14.3	20.0	17.1	19.3	20.0	22.9	27.7	21.7	24.0			28	Islet
PS36	32.3	0	0	0	0	0	0	0	0	0	1.1	6.6	5.4	5.1	6.0	99	BW↓
PS48	19.9	1.1	1.7	2.9	1.7	2.9	0	1.7	8.0	80	7.7	10.9	12.6	14.3		90	Clotting
PS49	20.9	0	1.1	0	0											26	BW↓
PS50	22.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	>162	
PS56	36.9	6.0	6.0	6.0	5.1	0	0	0	0	0	0	0	0	0	0	>78	
IP1	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	>125	

Islet, failure of glucose control attributed to loss of islet function; anastomosis patent through postoperative course and at postmortem examination.

BW, dog killed because of more than 20% loss of body weight with functioning device; anastomosis patent at postmortem examination; systemic bacterial sepsis present.

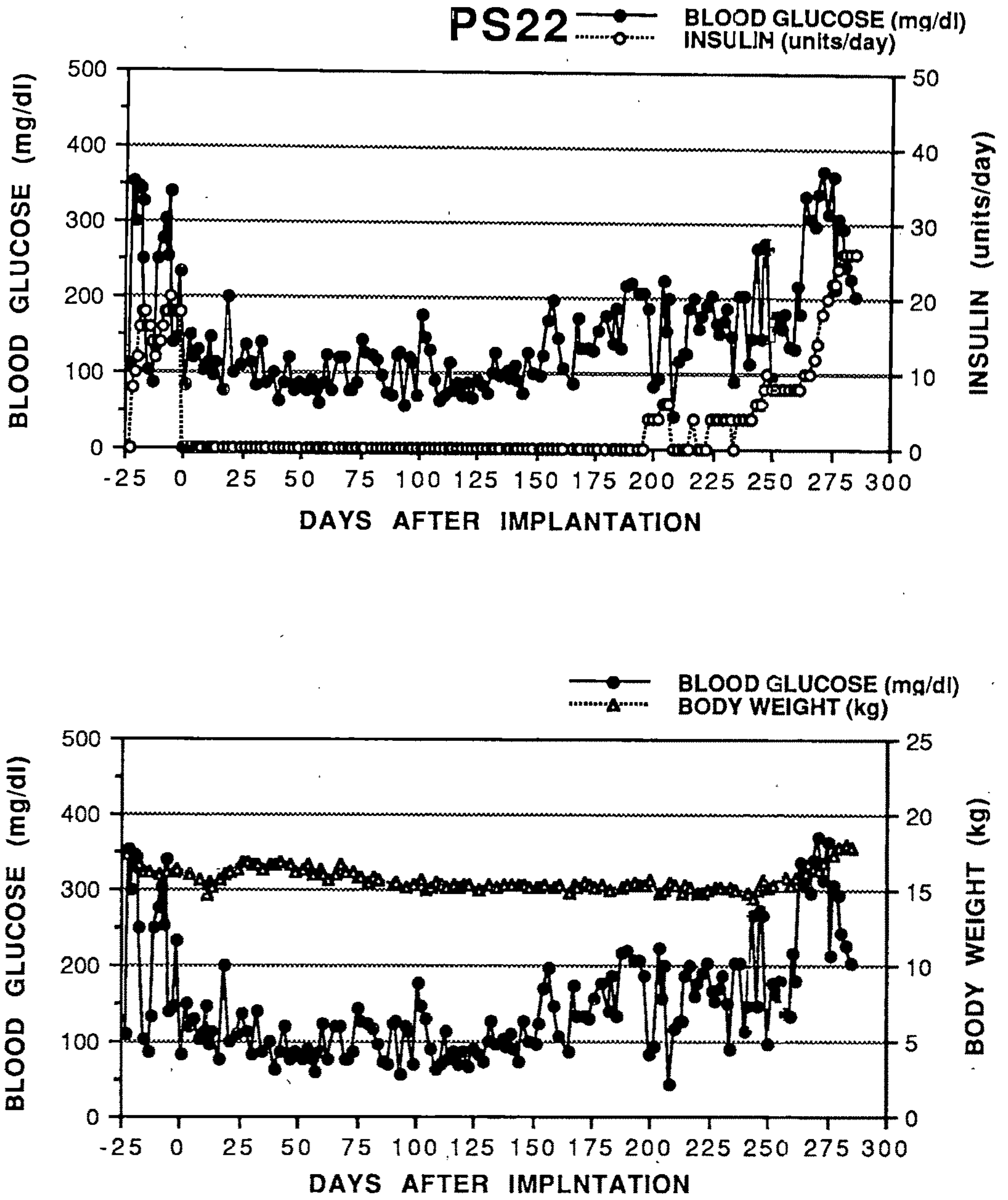


FIG. 5. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-de-vice implantation (allograft seeded) in dog PS22 (see text).

Less then 20% of the islets in both devices (see below) were found to be viable. Note should be made that the animal maintained his weight throughout the course of the experiment. Figure 6 illustrates the case dog PS23, which required approximately 20 units of insulin per day before implant. Again excellent, tight glucose control was maintained for the first 75 days with a blood glucose level at 100 mg/dL or less. At approximately 100 days after operative, tight glucose control was lost with varying glucose levels at or less than 200 mg/dL, although no insulin was required. On day 207 the animal became distended and eventually died on day 209 from small bowel obstruction secondary to adhesions to one of the devices. Weight was maintained normally throughout the postoperative course. On postmortem examination both devices were patent. Histologic examination revealed less than 20% of islets were viable. Figure 7 shows the postoperative course of dog PS50. This animal required 22 units of insulin per day before transplantation. Excellent glucose control was maintained for the first 40 to 50 days, followed by some deterioration for the succeeding 50 days, and spontaneous improvement for the next 60 days. This dog has not required insulin for more than 150 days after double-device implantation. Body weight is well maintained.

Figure 8 shows the postimplantation course of dog PS56 after double-device implantation. This dog initially required some insulin immediately after operation. How-

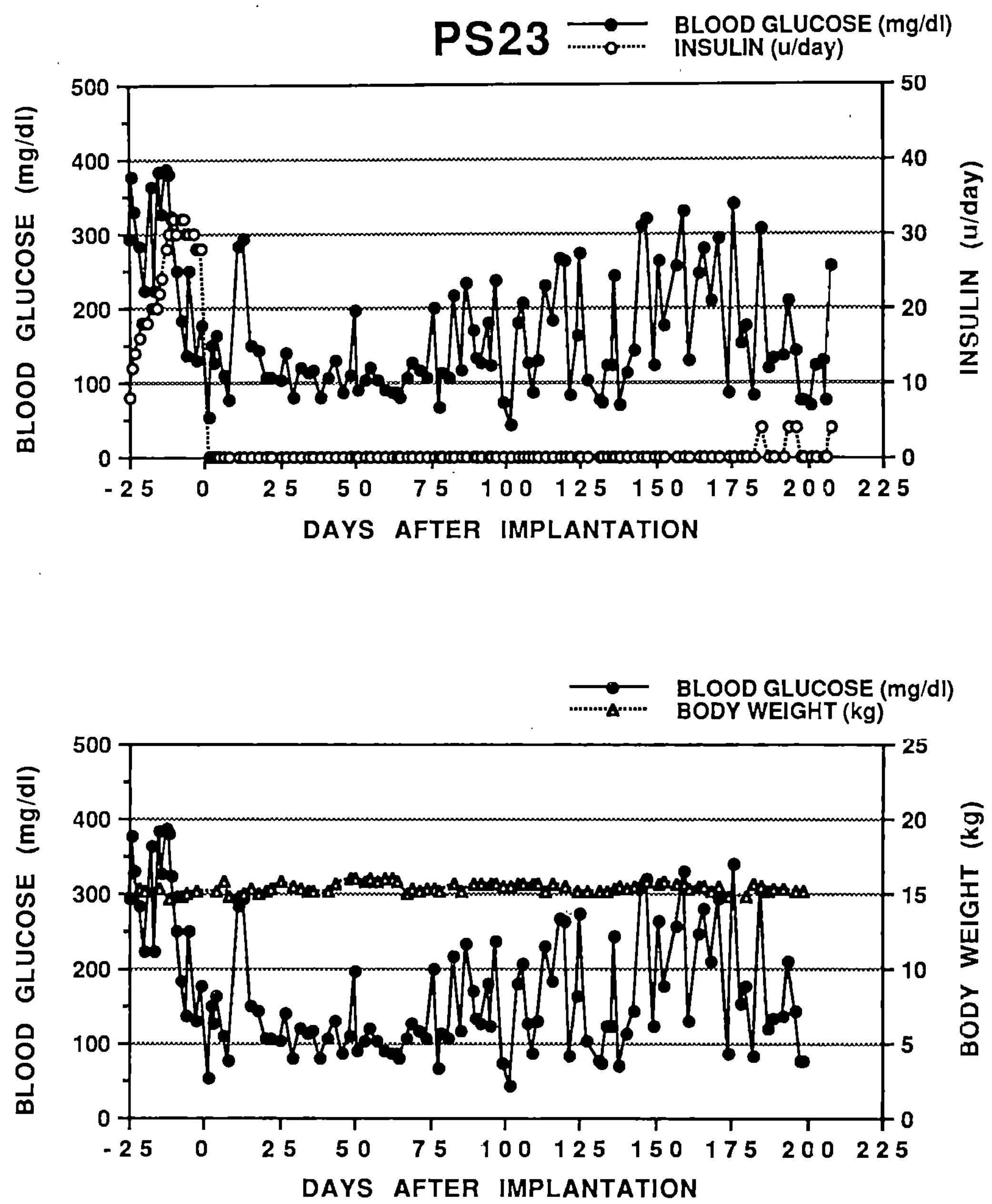


FIG. 6. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-device implantation (allograft seeded) in dog PS23 (see text).

ever, after approximately 3 weeks, blood glucose levels decreased and no exogenous insulin was required. This dog has not required insulin for 78 days after device implantation.

Xenograft-seeded Double Devices

Nine dogs have each received two implanted devices seeded with bovine islets and two dogs (PS43 and PS63) received devices seeded with porcine xenograft islets (10,000 to 15,000 islets/kg per device). Thus far we have had limited success (Table 4). Of nine dogs given bovine seeded devices, one had significant function at 106 days (PS29) while the other eight have essentially failed in that function (as defined as less than 50% of preimplantation insulin requirement) was only achieved for 3 to 16 days. Two dogs (PS42 and PS63) were implanted with porcine islets and functioned for 80 and 14 days, respectively.

Dog PS32 showed variable function for the first 3 weeks but lost audible bruit on day 21 and had occluded anastomoses at autopsy with 0% islet viability in both devices. PS32 had no real function and at autopsy had patent anastomoses but poor islet viability. Dog PS38 also had no real function and at autopsy showed thrombosed arterial and venous anastomoses with extensive infection in the device and zero islet viability in both devices. PS40 presumably had function for 2 to 3 weeks. At autopsy the right device was patent but the left was occluded with no islet viability. Dog PS46 functioned for about 2 weeks

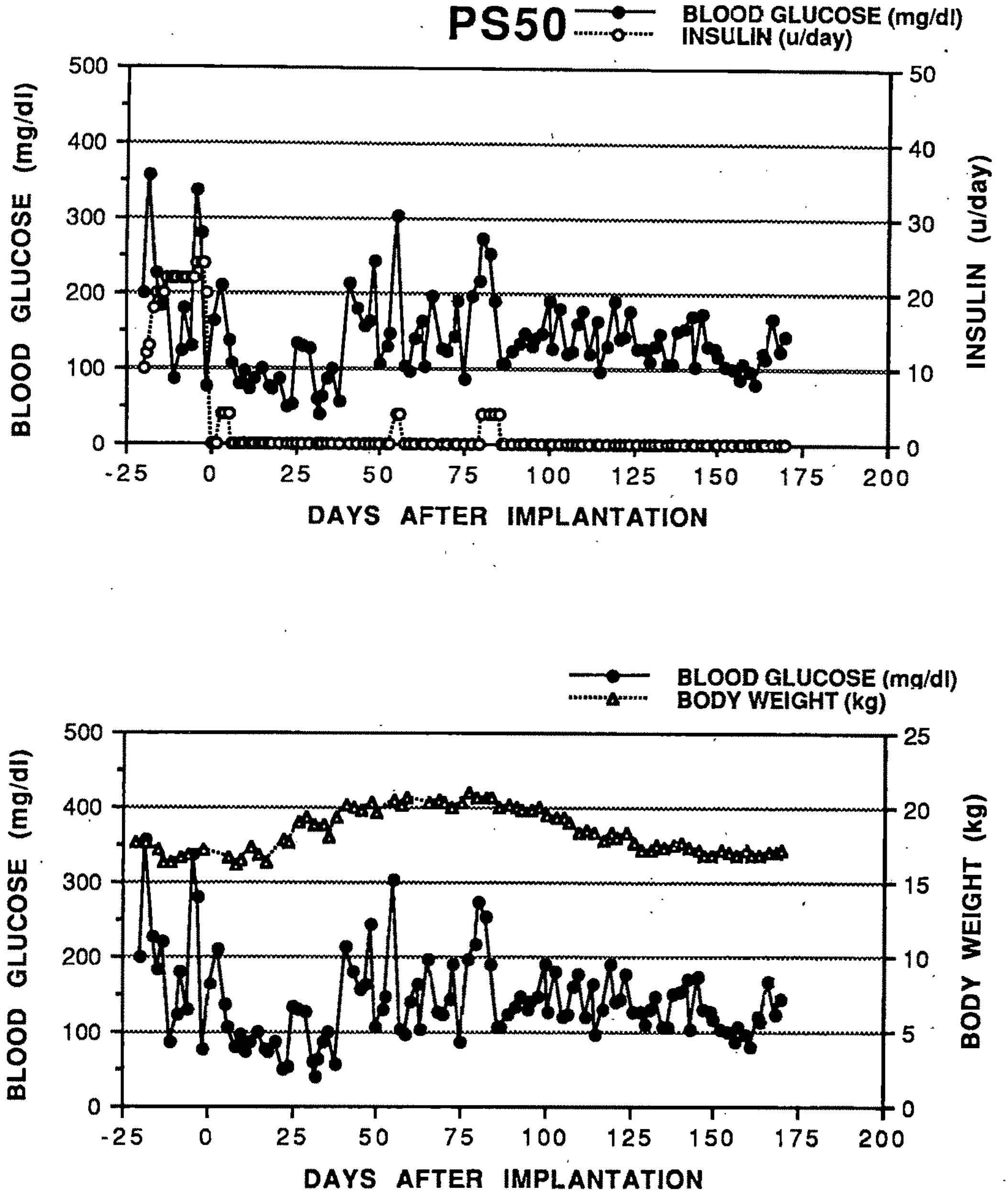


FIG. 7. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-device implantation (allograft seeded) in dog PS50 (see text).

and then lost function. At autopsy both devices were occluded and there was no islet viability.

Dog PS51 had occluded devices and no viability with extensive bacterial sepsis. Dog PS52 had patent devices but no islet viability in both devices as well as bacterial infection of both grafts and systemic sepsis. Dog PS53 had patent devices at autopsy but no islet viability in both devices and failure was attributed to lack of islet function. Dog PS63 had excellent function for 2 weeks but developed signs of pneumonia and had to be killed (autopsy results not available at this time).

Figure 9 illustrates the course of dog PS29, an animal that required 14 units of insulin per day before double-device xenograft implantation. For the first week after transplantation, no insulin was required followed by small requirements during the second and third weeks. The insulin requirement was then zero for approximately the next 50 days, with excellent fasting glucose control (less than 100 mg/dL). After 11 weeks insulin was again required with poor glucose control. After 100 days the insulin requirement exceeded 50% of the pretransplant dose and the device was considered nonfunctional. The weight of the dog was well maintained throughout this study. The animal was found dead in his cage on day 100 and autopsy failed to reveal a cause of death. Histologic examination of the device showed islet viability of less than

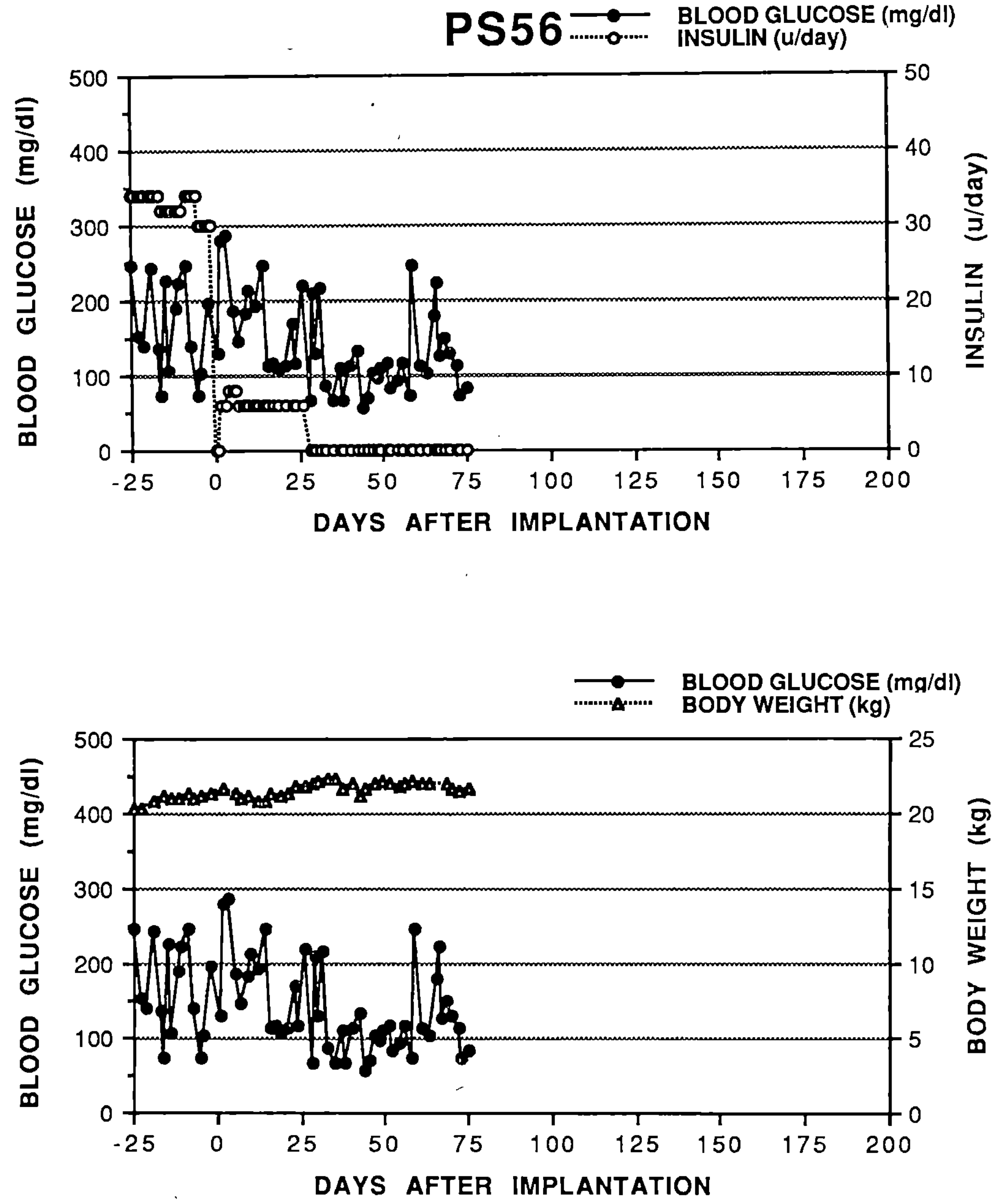


FIG. 8. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after device implantation (xenograft seeded) in dog PS56.

20%, although vascular anastomoses were patent. Because the insulin requirement was more than 50% before the animal's death and both devices were patent at autopsy, loss of islet function was the designated cause of failure. Figure 10 shows the postimplantation course of dog PS43. This animal required approximately 24 units of insulin per day before transplantation. For the first 10 weeks after transplantation, small amounts of insulin were required and the glucose control was acceptable but erratic. From days 75 to 100 after operation, insulin requirements increased to more than 50% of preoperative levels and glucose control deteriorated. The device was declared nonfunctional at day 80 (when more than 50% preoperative insulin was required). The device was recovered on day 142 and no viable islets were found to be present, despite the fact that the vessels were patent.

Metabolic Studies on Seeded Device Implanted Dogs

In our previous studies[19] of single-seeded devices of canine islet allografts in pancreatectomized diabetic dogs, data suggested that although both postprandial blood glucose levels and intravenous glucose tolerance tests were somewhat improved, they remained abnormal. Figure 11 is taken from a previous publication.[19] In both dogs (PS10 and PS7) whose cases are illustrated, the IVGTT improved

TABLE 4. *Insulin Requirement After Xenograft (Bovine and Porcine) Double Device Implantation*

		Mean Insulin Requirement (unit/day)														
		Postimplantation Week														
	Pre-Device −1 Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Function (days)
PS29	14.0	0	3.4	2.6	0	0	0	0	0	0	0	0	4.3	7.4	6.3	106
PS32	18.0	8.3	12.3	6.0												4
PS37	22.9	10.9	21.7	23.1	15.1	14.6	19.1	15.7	19.4	26.9	22.0	26.9	25.3	26.9	25.3	3
PS38	38.6	15.8														4
PS40	35.3	11.1	16.3	18.9	22.0	26.3	26.0	28.0	32.0							16
PS46	24.9	8.0	16.0	18.6	24.9	20.3	22.3	16.3	19.7	20.0	13.7	17.7				14
PS51	19.0	3.0														3
PS52	27.4	5.4	14.3	28.0												11
PS53	30.9	14.6	28.4													5
PS43	23.7	5.1	3.4	6.6	6.6	5.4	2.9	6.9	10.6	7.7	6.9	10.0	12.0	18.0	26.3	80
PS63	23.1	5.5	4.9													14

but never to normal. Also the abnormal postimplantation IVGTT stabilized for a period and then deteriorated. Because of difficulties of getting accurate postprandial studies in dogs (food must be given by gavage), only IVGTT studies have been performed in dogs that received double devices. Figure 12 illustrates the IVGTT in dog PS50, which has not required insulin for more than 160 days. On day 27 after operation, the IVGTT was abnormal but surprisingly improved. More than 120 days later, the IVGTT was still remarkably improved without further deterioration (as shown with dogs PS10 and PS57). Figure 13 shows a dog (IP-1) that had a double device implanted and after 2 weeks had a total pancreatectomy. After pancreatectomy the animal required little or no insulin. The animal has not required insulin for more than 100 days after pancreatectomy with well-maintained weight. Figure 14 shows that the IVGTT in this animal is also abnormal but significantly superior to that which one could expect in a completely pancreatectomized dog, as illustrated by the postpancreatectomy IVGTT (preimplantation) of dog PS50. Similarly dog PS56, which was free of insulin on day 70 after implantation, has an abnormal but significantly improved IVGTT at day 64. Although it is difficult to quantify, it is our impression that IVGTT studies in double-device allograft seeded dogs are more improved than in single-device animals and the IVGTT remains more stabilized.

Analysis of In Vitro *Insulin Output of Devices and Correlation with* In Vivo *Function as Determined by Reduction in Exogenous Insulin Requirement*

We recently reported the *in vitro* output of Hybrid Artificial Pancreas devices seeded with canine islet allografts. The devices were shown to maintain insulin output in the presence of a constant stimulatory glucose level (200 mg/dL). In perfusion studies the device could support islet function for more than 9 months, although the level of insulin output did show a decrease during the time of the culture. Data reported[20] from 18 devices maintained on culture for periods of 60 to 156 days (80 ± 5, mean + SEM) showed that insulin secretion peaked within the first 2 months of culture and then decreased slowly. Perfusion studies also demonstrated that islets responded to an increase in glucose concentration from 100 to 300 mg/dL with an increase in insulin secretion.

Table 5 lists the *in vitro* insulin output of canine allograft seeded devices at various times during perfusion. Because insulin output peaked in the 2 months after initiation of perfusion, correlation was made with average daily outputs during the first 30 to 60 days only.

It is important to re-emphasize that for each dog in the double-device allograft group (shown in Table 3) a device was seeded with a similar number of islets from the same islet preparation. This device was perfused *in vitro* beginning the same day the other two devices were implanted surgically. To estimate the amount of insulin that might be secreted *in vitro* by the two implanted devices, the output of the single-perfused device was simply doubled. Dogs PS22, PS23, PS30, PS48, PS49, and PS50 required little or no insulin after implantation through the first 30 to 60 days and the estimated *in vivo* output of two devices (double the *in vitro* output of a single device) correlated well with the preoperative insulin requirement. Data from dog PS28 also correlated remarkably well. Dog PS24 required almost 14 units per day and thus correlated with a estimated output of two devices of 11 units and a postoperative insulin requirement of 26.6, *i.e.*, estimated *in vivo* output plus postoperative exogenous insulin equalled the preoperative insulin dose. On the other hand, dogs PS26, PS36, and PS56 showed less correlation and the preoperative requirements were larger than estimated *in vivo*

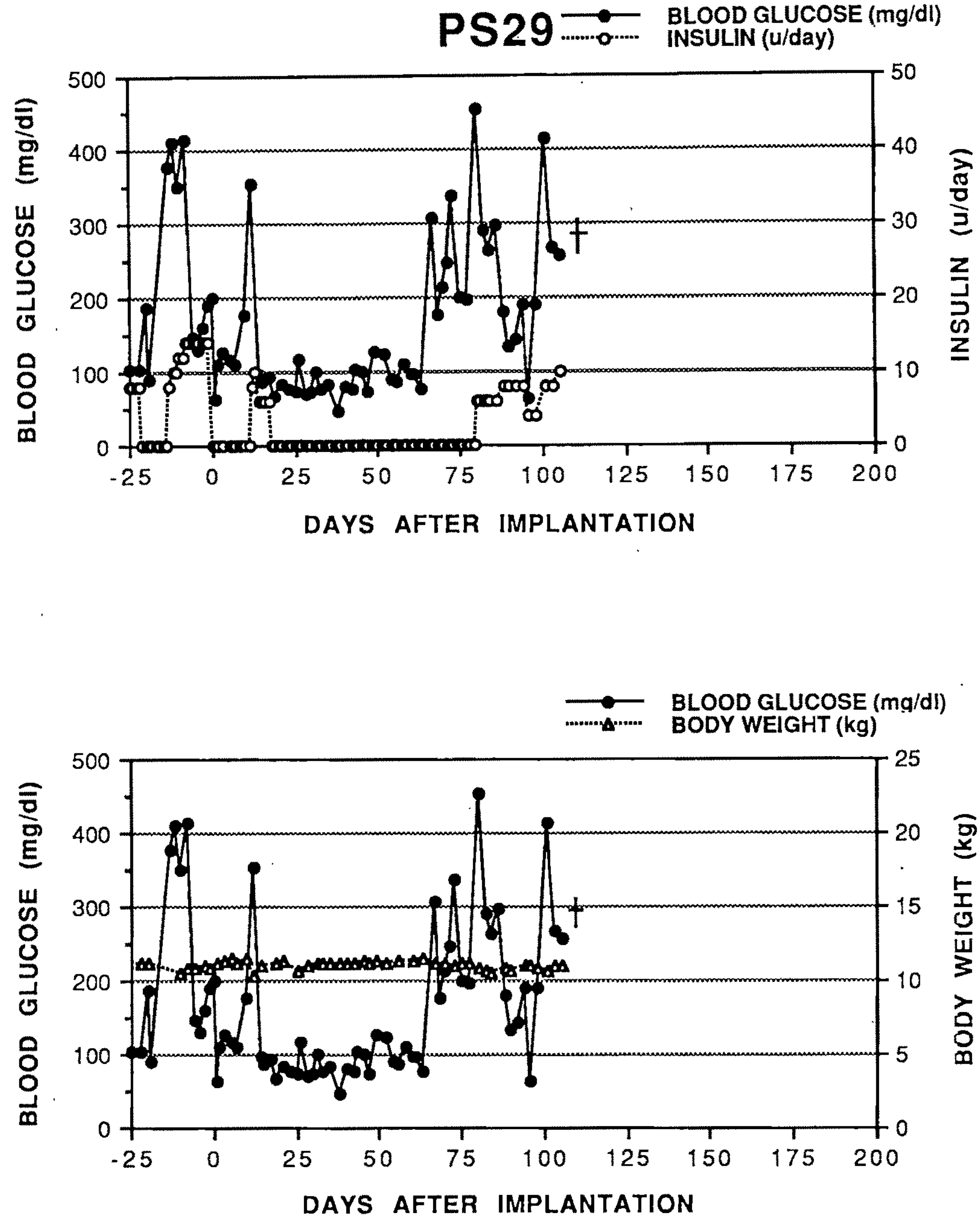


FIG. 9. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-seeded device implantation (xenograft seeded) in dog PS29.

output of two devices (PS26 and PS36) and exogenous insulin requirements were significantly less than would have been expected.

Table 6 presents a similar analysis for the xenograft animals. It is of interest that the two long-term xenograft dogs (PS29 and PS43) had good correlation between the estimated *in vitro* insulin output and the preoperative insulin requirement. Data was not available for dogs (PS38, PS51, PS52, and PS63). Few conclusions can be made from the data from the other dogs because their survival was so short relative to length of time of *in vitro* perfusion.

Analyses of Device Patency, Histologic Islet Viability, and Causes of Failure After Double-Device (Allograft) Implantations

Table 7 presents a summary of the length of function, anastomotic patency, estimate of percentage of islet viability on histologic analysis, and cause of failure for all dogs receiving double device (allograft) implantations. Dogs that lost function had devices surgically removed and subjected to histologic analyses. Dogs were subsequently killed and a complete autopsy was performed on

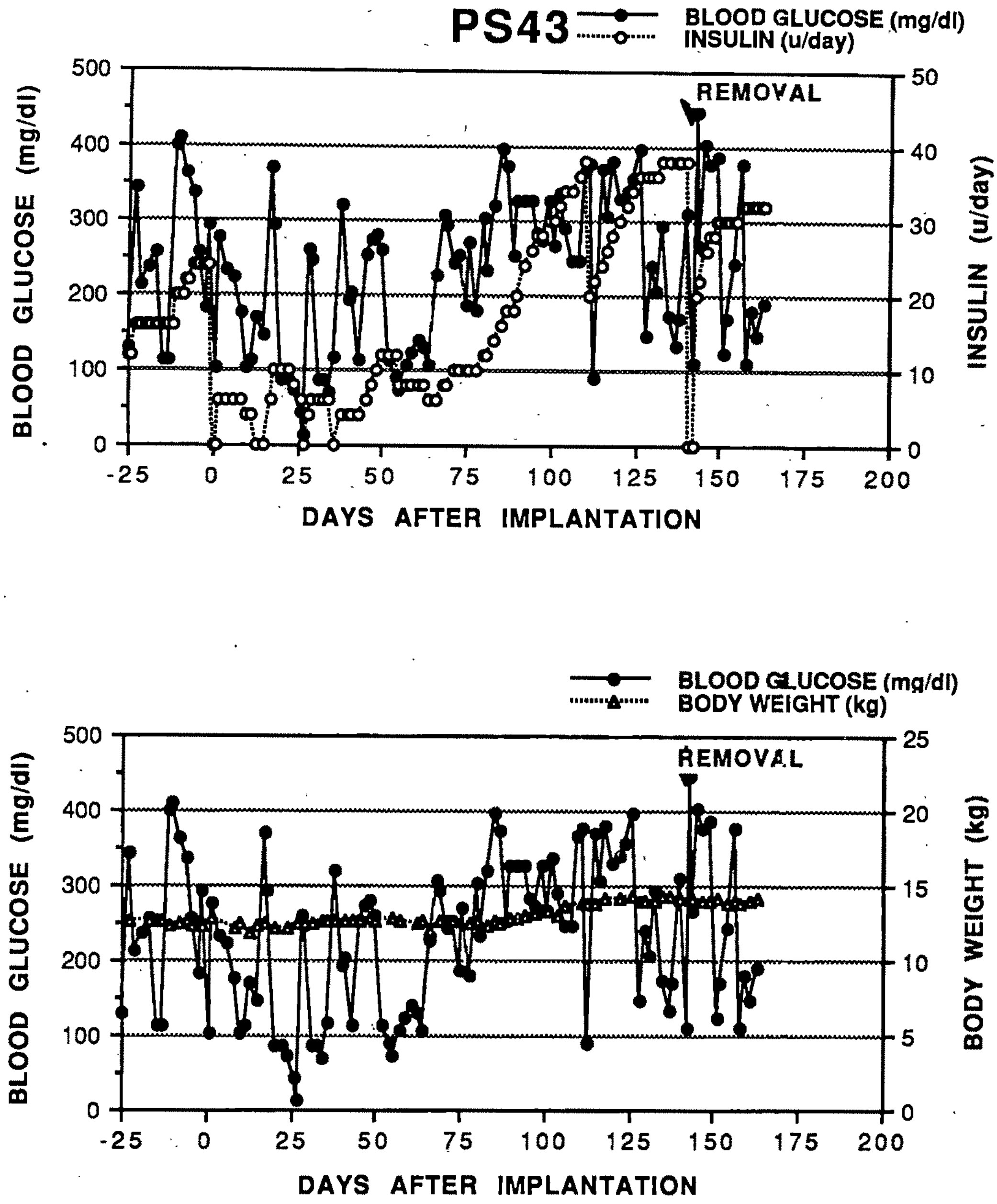


FIG. 10. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-seeded device implantation (xenograft seeded) in dog PS43.

each one after sufficient time passed to determine if additional exogenous insulin requirements developed after device removal. Some dogs were killed because they lost up to 20% of their body weight, even though the devices were functioning (dogs PS28, PS30, PS36, PS49). Histologic estimates of islet viability were performed by first examining all islets in several representative sections for characteristics of viability (see Materials and Methods). The number of islets showing 0% viability, 25% viability, 50% viability, 100% viability, and so on was determined and a composite score for total islet viability for the entire device was given.

It is clear that device thrombosis is not a major cause of device failure. Of 20 devices examined in this series of double-device implants, only three were thrombosed. An additional three dogs (PS50, PS56, 1P1) have devices still functioning (audible bruits and good glucose control) so it is likely that their device anastomoses are patent. Therefore it is likely that 22 of 26 devices in this series have remained patent during the study period (85% patency). Four dogs (PS28, PS30, PS36, PS49) were killed because of 20% loss of body weight with functioning devices. These dogs had essentially all patent anastomoses, except for the left device in PS28. It is of interest that these dogs had devices that generally contained more viable islets than the other dogs who had been functioning longer but that had subsequently lost function. The dogs that lost function with patent anastomoses (PS22, PS24, PS26, PS35) and the dog who died with a functioning device(s) (PS23) had low levels of viable islets.

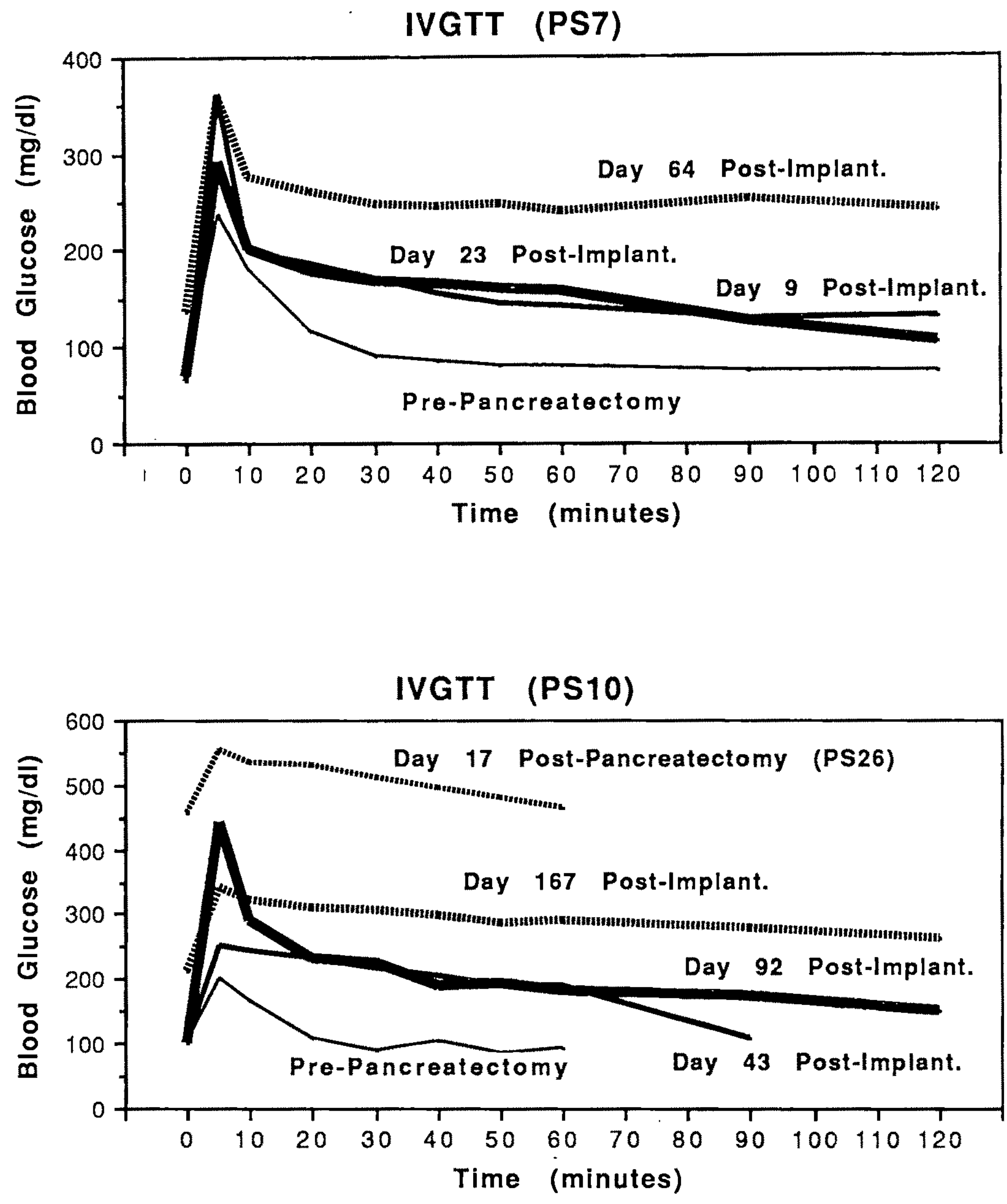


FIG. 11. Intravenous glucose tolerance test in dogs PS10 and PS7. An IVGTT of pancreatectomized dog (PS26) before device implantation is shown with IVGTTs of PS10. Deterioration of IVGTTs in both dogs is clearly shown with successive IVGTTs performed serially after implantation.

Table 8 presents a similar analysis of the small group of xenograft dogs. Thrombosis of devices was present in four dogs (PS32, PS38, PS40, PS51), three of which had very significant systemic infection. Islet viability was poor in all dogs, although dogs (PS29 and PS43) with the longest function still had some viable islets when they were killed.

Histologic Examination of Devices

Histologic examination of devices recovered at various times after implantation has revealed several important points. First the devices with intact membranes never showed any type of cellular infiltration within the chamber. Rather islets are found dispersed throughout the chamber with varying degrees of viability. Figure 15 shows a cluster of islets in the chamber of dog PS19 with excellent viability and insulin content. Note that no inflammatory cells or cells associated with immunologic rejection are apparent. Thus histologic evidence for a cell-mediated rejection or an antibody-mediated rejection is not readily identified. Figure 16 shows a group of canine islet allografts that are of poor viability (Fig. 16A), and a closer magnification of a different section (Fig. 16B) shows islets of excellent viability next to vacuolated areas in the surrounding device matrix that contained islets that have become necrotic. Figure 16 also shows that the semipermeable membrane surface facing the blood stream (non-islet side) has only a thin layer of fibrin deposit but no fibrosis. No cellular elements are discernible in the layer of fibrin deposition. Figure 17 shows a section for a xe-

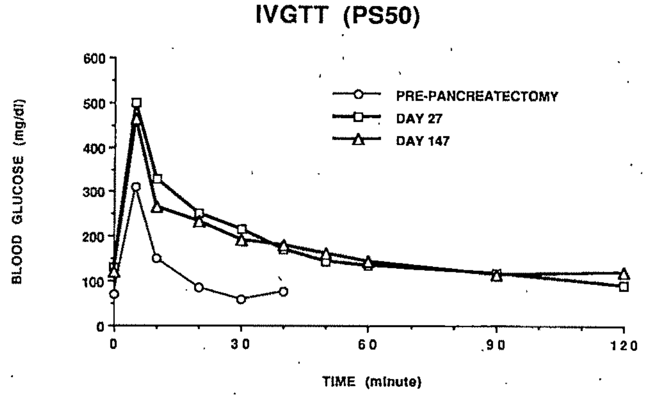


FIG. 12. Intravenous glucose tolerance tests in dog PS50, which has not required insulin for more than 160 days. The IVGTT, although abnormal compared to the prepancreatectomy studies, is significantly improved on day 27 after implantation and that improvement was maintained through day 147 after implantation.

nograft dog. These sections are of interest in that they show no inflammatory cell infiltration even around the viable islets close to the semipermeable membrane.

Discussion

The Hybrid Artificial Pancreas incorporates a single, large-bore, hollow fiber membrane that is connected to commercially available vascular PTFE grafts of the same diameter (5 to 6 mm, ID). The technical details of the development of this prototype design have been described and discussed in detail.[19] The current experiments have been devised to test further the performance and efficacy of the artificial pancreas as an immunoexclusion device for islet transplantation.

The experiments in which unseeded devices were surgically implanted and followed for up to 20 months clearly

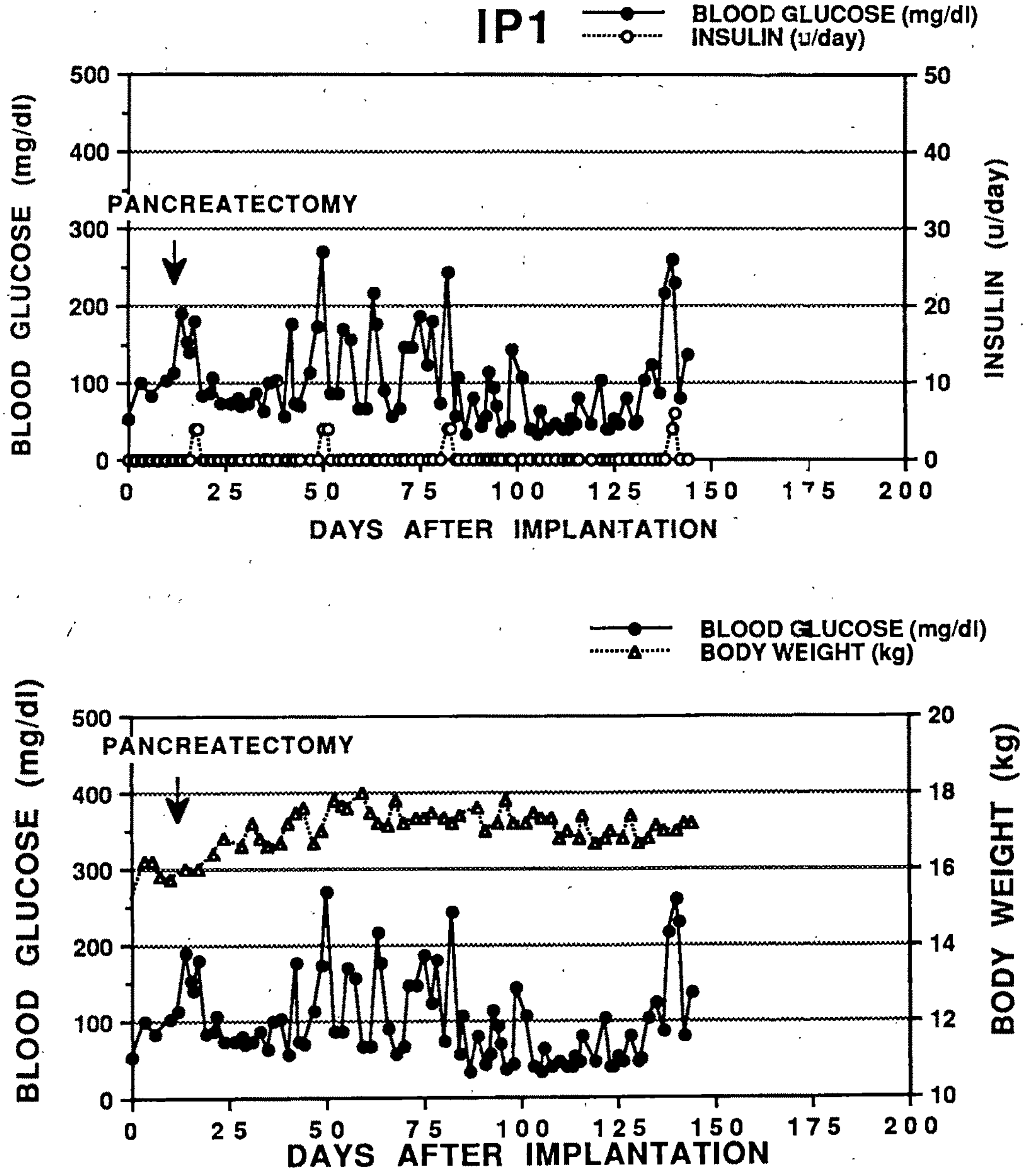


FIG. 13. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-seeded device implantation (allograft seeded) in dog IP-1, which has not required insulin for 100 days (see text).

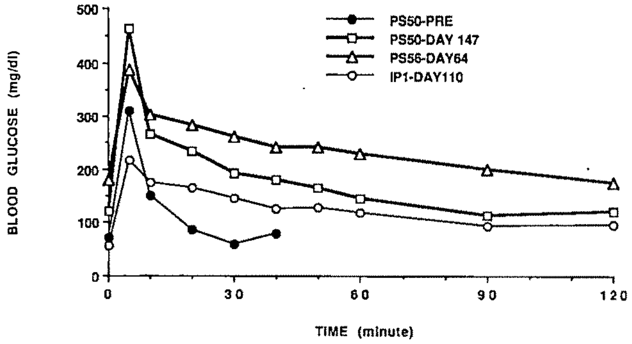


FIG. 14. Intravenous glucose tolerance tests in dogs PS50, 56, and IP-1 at various times after implantation of double devices (allograft seeded).

TABLE 6. *Correlation of Insulin Requirements After Double Xenograft Seeded Device Implantation and* In Vitro *Insulin Output*

				In Vitro Output	
Dog	Preimplantation Daily Insulin Req.	Average Daily Insulin Req.	Up to Day	Single Device	Double Device
PS29	14.0	0.7	60	8.7	17.4
PS32	18.0	8.9	20	4.7	9.4
PS37	22.9	18.5	60	7.8	15.6
PS38	38.6		Data not available		
PS40	35.3	17.1	30	4.7	9.4
PS43	23.7	5.6	60	8.4	16.8
PS51	19.0		Data not available		
PS52	27.4		Data not available		
PS53	30.9	21.5	30	5.1	10.2

demonstrate the potential long-term patency of the current device. The devices of 10 of 12 of the dogs in group I (unseeded device) eventually occluded. Their implantations may have failed because they were performed without benefit of aspirin therapy, an end-to-end anastomosis was used, and a certain amount of technical learning with the device was necessary. Devices remained patent in seven of eight group II unseeded dogs more than 500 days after end-to-end anastomoses and administration of low-dose aspirin therapy. Clearly the semipermeable membrane is not thrombogenic in this setting. Also any defect in the semipermeable membrane would cause bleeding into the chamber with collapse of the membrane leading to the hollow fiber membrane tube and graft thrombosis. That these devices remain patent attests to the fact that the membrane can withstand arterial pressure for prolonged periods without losing its physical integrity. Furthermore the long-term unseeded device dogs remain healthy thus far; they clearly tolerated the long-term arteriovenous shunt without cardiac or other difficulty. Finally it should be noted that a 5- to 6-mm PTFE graft was used on these prototype devices, a size that is commonly used clinically in humans. These grafts stayed open in 15- to 20-kg dogs. Thus it is highly probable that long-term patency in humans will be no problem.

The model of total pancreatectomy to induce diabetes used in these experiments is a difficult one but a highly effective one. The fact that less than 1.5% of dogs subjected to surgical total pancreatectomy fail to become diabetic attests to its efficacy. Also no dogs (66 of 67) that became hyperglycemic after pancreatectomy became spontaneously normoglycemic. The model does have some drawbacks in that despite nutritional supplements, some animals sustain weight loss. Also a number of animals lost significant amounts of body weight after device implantation and the experiments had to be terminated despite the fact that the devices were functioning normally. The diminished nutritional reserve of some dogs certainly may have contributed to susceptibility to infection, which also necessitated killing of several dogs with functioning devices.

Experiments with allograft seeded devices clearly emphasize that the Hybrid Artificial Pancreas can function

TABLE 5. *Correlation of Insulin Requirements After Double Allograft Seeded Device Implantation and* In Vitro *Insulin Output*

				In Vitro Output	
Dog	Preimplantation Daily Insulin Req.	Average Daily Insulin Req.	Up to Day	Single Device	Double Device
PS22	18.3	0.0	60	7.2	14.4
PS23	20.6	0.0	60	14.0	28.0
PS24	26.6	13.7	60	5.5	11.0
PS26	27.7	0.0	60	8.3	16.6
PS28	22.9	2.3	30	14.0	28.0
PS30	16.3	0.0	30	6.9	13.8
PS35	29.7		Data not available		
		0.0			
PS36	32.3	1.8	30	8.6	17.2
PS48	19.9	0.3	30	10.8	21.6
PS49	20.9	0.0	30	8.0	16.0
PS50	22.1	0.0	60	13.1	26.2
PS56	36.9	5.8	30	4.4	8.8

TABLE 7. *Anaysis of Device Patency, Islet Viability, and Causes of Failure After Double Device (Allograft) Implantation*

		Device Patency		Islet Viability (%)		
Dog	Function (day)	R	L	R	L	Cause of Failure
PS22	263	+	+	15	25	Islet
PS23	209	+	+	10	15	Ileus
PS24	64	+	+	10	10	Islet
PS26	169	+	+	25	15	Islet
PS28	51	+	−	40	0	↓BW (sepsis)
PS30	29	+	+	70	30	↓BW (sepsis)
PS35	29	−	+	0	NA	Islet
PS36	99	+	+	<15	NA	↓BW (parasites)
PS48	72	−	−	0	0	Thrombosis
PS49	26	+	+	25	15	↓BW (sepsis)
PS50	>162					
PS56	>79					
IP1	−125					

TABLE 8. *Anaysis of Device Patency, Islet Viability, and Causes of Failure After Double Device (Xenograft) Implantation*

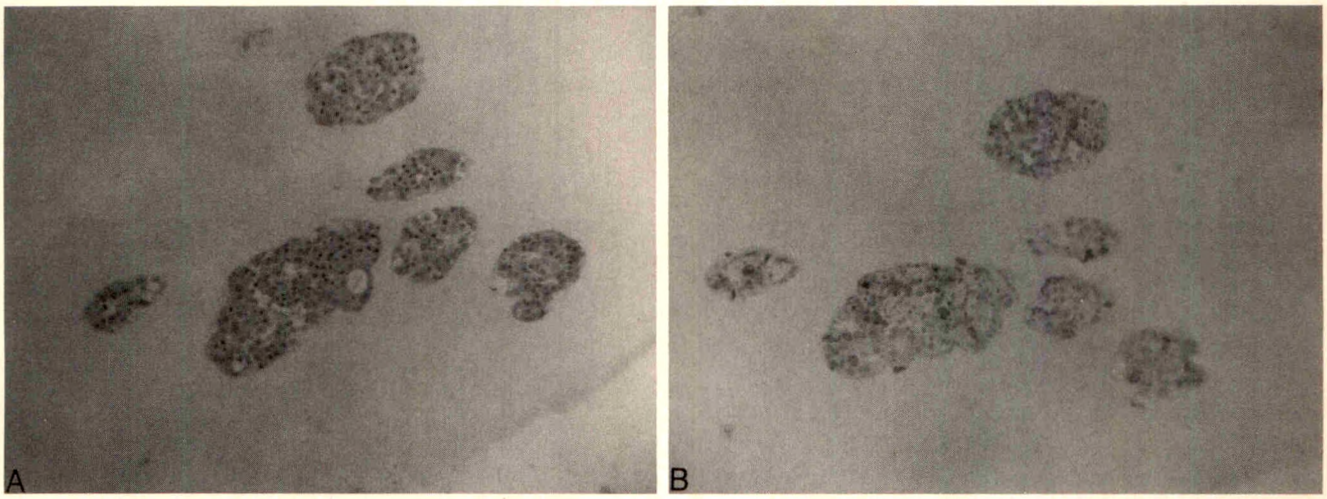
Dog	Function (day)	Device Patency R	Device Patency L	Islet Viability (%) R	Islet Viability (%) L	Cause of Failure
PS29	106	+	+	25	10	Islet
PS32	4	−	−	0	0	Thrombosis
PS37	3	+	+	20	20	Islet
PS38	4	−	−	0	0	Infection (thrombosis)
PS40	16	+	−	NA	0	Thrombosis
PS46	14	+	+	0	0	Islet
PS43	80	+	+	12	7	Islet
PS51	3	−	−	0	0	Infection (thrombosis)
PS52	11	+	+	0	0	Islet, infection
PS53	5	−	+	0	0	Islet
PS63	14	+	+	NA	NA	↓BW

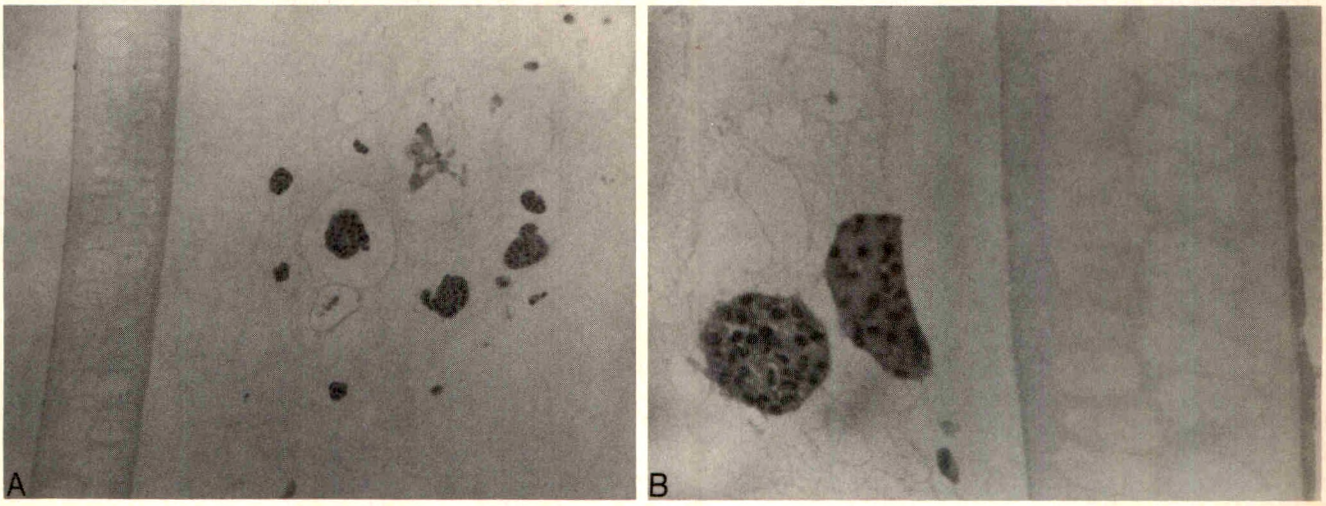
in large animal species to facilitate blood glucose control. The extraordinary survival of totally pancreatectomized dogs with good blood glucose control without administration of insulin for extended periods after transplantation of islet allografts in this device is clearly encouraging. Canine islet allografts transplanted without immunosuppression or with large doses of multiple immunosuppressive drugs are invariably rejected in a few to several days.[13] Not only have islets in the Hybrid Artificial Pancreas survived without immunosuppression but they functioned for the longest periods thus far achieved in islet allotransplantation in large animal models. Four of fifteen single-seeded devices functioned more than 90 days with significant reduction of exogenous insulin requirements after implantation compared to 7 of 13 double-device dogs. Seven of fifteen single-device dogs showed little or no function while essentially all the double-device dogs had definite function. The most probable explanation for this is that single devices did not provide sufficient insulin output to alter glucose control measurably. Analysis of the *in vitro* insulin output by perfused devices in previous studies[20] and in these current experiments suggest that the average daily insulin output for one device is approximately 10 units of insulin or less. These findings support the inadequacy of single-device *in vivo* insulin output to control blood glucose in most dogs with this severe model of diabetes. It should be noted that the one single-device dog that lived more than 280 days (PS10) had preimplantation insulin requirements of approximately 11 units per day.

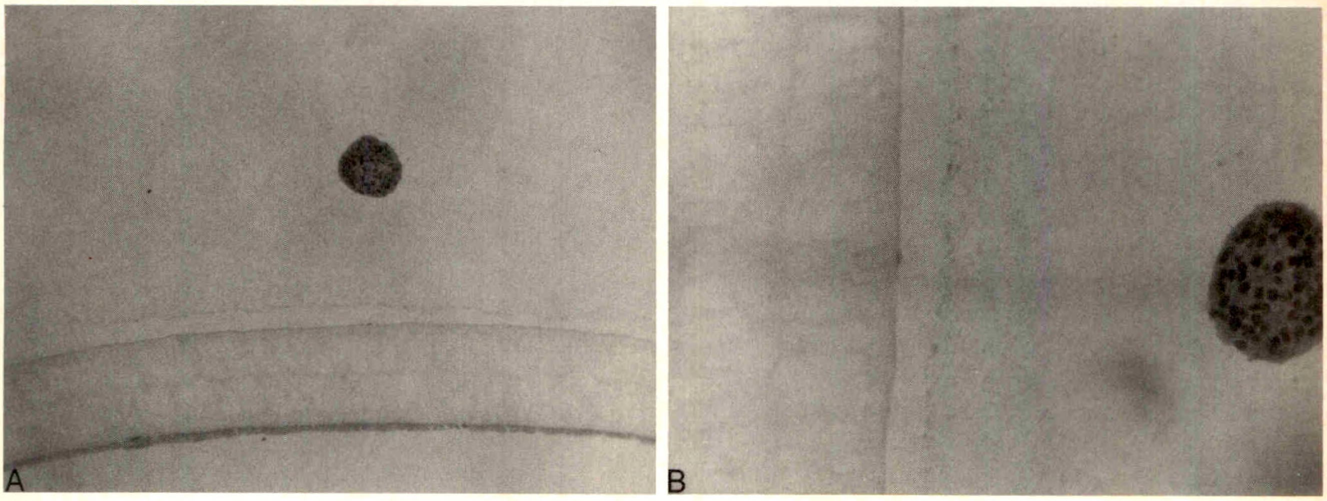
The current studies have permitted an extended analysis of the reasons for failure of the device to control blood glucose levels in these diabetic animals. It is noteworthy that failure in the single-device animal was attributed to clotting in only 3 of 15 dogs and device failure in only 2 of 15 dogs. As best as could be determined, an important cause for failure was related to loss of islet function and/or viability. Thrombosis was a cause of failure in only 1 of 13 dogs in the double-device allograft group and there were no device failures. Again the major cause for loss of device effectiveness was islet function loss.

In the single-device allograft studies,[19] IVGTT tests in dogs with functioning devices are improved over prepancreatectomy levels but still are not normal. Also postprandial blood glucose levels in such dogs are also significantly elevated for several hours. The abnormal IVGTT is probably not attributable to a slow response of the device to the increased glucose because of the large islet chamber volume (approximately 5 to 6 mL). *In vitro* studies of insulin secretory dynamics suggest that insulin output into the perfusate begins to increase 15 to 20 minutes after glucose stimulation. The problem appears to lie in the inadequate overall insulin secretion of these devices to manage hyperglycemic stress. One obvious cause is that in a single device, the device can be seeded with only a limited number of islets that produce insufficient amounts of insulin in response to carbohydrate stress. Alternatively the microenvironment surrounding the islets may still not be optimal and could contribute to loss of function and viability. In either case it is likely that insulin secretion is already close to maximal with these devices and excess carbohydrate loading thereafter results is sustained hyperglycemia. Evidence presented in these double-device allograft experiments support this conclusion. Double-device dogs have better long-term control of fasting blood glucose and a higher percentage of these dogs show significant function. Also IVGTT studies in these dogs appear to be more improved compared to postpancreatectomy tests and remain improved for longer times.

The studies of serial fasting blood glucose levels in dogs with two functioning devices demonstrated an excellent control of blood glucose for approximately 100 days. Not only was no insulin required but the blood glucose levels clustered around 100 mg/dL. Thereafter blood glucose control became less tight, with glucose levels increasing to the 150 to 250 mg/dL range but with little or no insulin required. Eventually most of these dogs appear to lose islet function and the device fails. This loss of islet function can be secondary to insufficient islet mass persisting in the device perhaps from loss of viability secondary to postprandial hyperglycemia and/or initial injury to the islets in their preparation and extraction. Loss of islet viability demonstrated by histologic analysis of the removed devices could also be due to inadequate nutrition within the microenvironment surrounding the islets. Loss of islet viability occurs in microencapsulated devices[17] secondary to pericapsule fibrosis. Fibrosis does not seem to occur on the blood stream side of the semipermeable membrane in the Hybrid Artificial Pancreas device. Clearly more sophisticated and detailed examination of the semipermeable membranes of removed devices is required, including electron microscopy studies, and so on. Also development of anti-insulin antibodies could impair function of device-







FIGS. 15A and B. (A) Histologic section of a device removed from dog PS19 showing a cluster of viable islets with no surrounding cell infiltration. H&E stain (×160). (B) The same section stained for insulin (×160).

FIGS. 16A and B. (A) Section of a device taken from dog PS25 showing islets of poor viability. The semipermeable membrane is superior (H&E, ×160). (B) Higher-power magnification of an area of device from dog PS25 showing viable islets next to a vacuolated area in the device matrix in which the islets have become necrotic and lyzed (H&E, ×400).

FIGS. 17A and B. Section of a device taken from a xenograft dog that had short-term partial function; the device was removed at day 18. The islet is viable and there is no surrounding cellular infiltrate, even though the islet is very close to the membrane. (A) H&E (×160); (B) (H & E, ×400).

produced insulin, although this is doubtful in the allograft situation. Another possibility is that insulin output is really adequate but some type of peripheral insulin resistance develops. Finally the possibility exists that loss of islet viability and function may be due to some type of rejection response. Against conventional rejection is that lack of demonstrated cellular infiltrate at any time in the removed devices or histologic examination. Destruction by some type of immunoglobulin and/or complement mechanism is doubtful but immunohistochemical studies and analysis of removed devices is clearly indicated. Similarly a lymphokine-mediated destruction should be ruled out by appropriate studies. Thus far detailed studies on the host immune response (except for histologic examination) have not been done in dogs with functioning or failed devices.

The studies in which xenograft islets were transplanted without immunosuppression in the hybrid artificial pancreas were not as successful as the allograft studies. Nevertheless the survival of the two xenograft transplanted dogs without immunosuppression represents the longest large animal islet xenograft survival thus far accomplished in any large animal model with or without immunosuppression. Indeed large animal islet xenograft survival is usually measured in hours even with immunosuppression.[23] The xenograft device studies were clearly limited by loss of islet viability and infection. Bovine xenografts were harvested at local abbatoirs under less-than-optimal conditions with significant warm ischemia times. Our future experiments will use surgically harvested xenograft pancreata with little or no warm ischemia time. Significantly better results are to be expected.

Subtherapeutic levels of insulin output by single devices is a limitation of the Hybrid Artificial Pancreas.[20] Redesign of the prototype device to increase the membrane surface area and the size of the available islet chamber should permit increased insulin output from single devices. Single devices with higher insulin outputs should also have increased insulin output with carbohydrate stress and improved postprandial glucose control. Nevertheless failure to achieve adequate control of postprandial blood glucose levels during times of carbohydrate stress would not necessarily preclude use of the hybrid artificial pancreas. For example the device could also be used to provide a steady state of insulin output, which would require supplemental exogenous insulin during times of carbohydrate stress. Thus the overall management of the difficult patient could be made easier. Long-term islet viability and function remains an important problem but should be improved by less traumatic isolation techniques and by changes in the microenvironment of the islet chamber. Another possible means of managing loss of islet viability is the capacity to reseed a functioning device that has lost function. It is planned that the device will be implanted in an anatomic location where access to the reseeding parts would easily be accessible through a local anesthetic procedure. Reseeding experiments have been started in dogs.

Significant progress has been achieved in developing the Hybrid Artificial Pancreas. Clinical application in the future using xenograft islets is a reasonable goal and possibility.

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DISCUSSION

DR. CLYDE F. BARKER (Philadelphia, Pennsylvania): New things often seem impossible until someone makes them work. Dr. Monaco has shown us this morning that something that many of us thought was impossible actually does work. I look forward to the paper on clinical use of this method, which he has promised to deliver at the Association meeting in Baltimore, 2 years from now.

Other than standard insulin injections, at least five approaches to the control of diabetes are under active experimental study: (1) transplantation of vascularized pancreas allografts in humans, which currently has approximately a 75% success rate for 1 to 2 years; (2) free transplantation of isolated pancreatic islets, which is successful in experimental animals but so far has produced disappointing clinical results. Only a handful of patients have been able to discontinue insulin even briefly after this procedure, and probably only one now is off insulin; (3) freely transplanted isolated islets enclosed in tiny semipermeable capsules, which sequester them from the immune system; (4) continuous delivery of insulin by external pumps or internally implanted ones; (5) the ingenuous system that Dr. Monaco has described this morning, which in a sense is a combination of the insulin pump and the isolated islet transplant.

There are several additional approaches that appear to be even further from clinical application. One is to take nonendocrine cells (such as fibroblasts) from a diabetic, program them by genetic engineering to secrete insulin, and then autotransplant these cells back into the same individual. Although these cells would not be rejected, another difficult obstacle would be endowing them with glucose-sensing ability to deliver the proper amount of insulin. Finally I should mention an approach on which we presented a paper to the Association several years, namely, prevention of diabetes by manipulation of the immune system, in this case by bone marrow transplantation. I believe some such approach eventually will be successful. I do not think this will be ready for presentation at the Baltimore meeting 2 years from now, but I think it will come.

In the meantime the method described by Dr. Monaco this morning may turn out to be the first to show an acceptable clinical result. Yet in some respects this method necessitates overcoming a biologic problem as difficult as allograft rejection; namely, the tendency for blood to clot on nonbiologic surfaces and the body's inclination to cover implanted foreign materials with cells and fibrous tissue. The latter process may tend to prevent insulin secreted by Dr. Monaco's islets from reaching the bloodstream rapidly enough to maintain continuous normoglycemia. Nevertheless it is extremely encouraging that Dr. Monaco has been able to accomplish the results he described today.

I have several questions. Because the real goal is to prevent the complications of diabetes by better control of hyperglycemia than simple insulin injections allow, is there evidence that such complications are less in those dogs that have maintained patent devices for many months than with simple insulin injections? Although the fasting blood sugar is normal in Dr. Monaco's best animals, the glucose tolerance is not. This is discouraging because it seems likely that virtually normal fasting and nonfasting blood sugar must be maintained to prevent diabetic complications.

A related question is whether Dr. Monaco can determine in advance the proper number of insulin secreting cells to be implanted. Presumably overdosing is not a problem, as it is with the insulin pump. But can the device be evaluated *ex vivo* before implantation to determine whether one or two or three such devices might be necessary?

Is there evidence of any immune response at all? If not one would expect xenografts to work as well as allografts, and clearly they did not. Perhaps the obligatory warm ischemia mentioned is the explanation. Yet Craig Reckard reported that several hours of warm ischemia of the pancreas do not compromise isolation of viable isolated cells, at least in the rat.

Finally, is there any indication that other biologic problems, such as fibrosis, thrombosis, or intimal proliferation on the inside of the devices, will prejudice the excellent results that we all hope will occur?

DR. MARK A. HARDY (New York, New York): Because this approach appears to perhaps permit the use of xenografts, it certainly might be practical for eventual clinical application, where the shortage of human donor pancreases would really never allow us to transplant organ-needy diabetic patients. And I too look forward to this Baltimore meeting and the paper in clinical situations.

But their approach raises several physiologic and technical questions, which I would like Dr. Monaco to address. One obviously is the matter of clotting, and does he have any data in terms of platelet adhesions and fibrin deposition in these devices, so that we can get some more objective criteria in terms of the long-term survival of these devices, particularly in another animal, perhaps, because the dog blood does not clot too well, as many vascular surgeons who have done experiments in this area know.

The other question that was alluded to by Dr. Barker I also would like to ask, and that is the matter of sensitization. Are there any data on the production of antibodies by these animals or cytotoxin T cells to the target allograft, or particularly to the xenograft? Are these animals in fact sensitized?

I realize that the histology is the purest and cleanest and lymphocytes do not go in there, but there are other antibodies.

And finally, the matter of the physiologic function regarding normalization. Is there any glucagon secretion? What is the timing in terms of the responses? And has the device been tried in any spontaneously diabetic animals where the matter of the autoimmune destruction is very important, as both Dr. Barker's group and my group have shown in the past?

DR. ROBERT J. CORRY (Iowa City, Iowa): I think the majority of my questions have been addressed by the previous speakers, but I wanted to compliment Dr. Monaco on this intriguing report. Because there are so many complications with the whole organ grafting, this technique will be very important in the future.

I wanted to ask one question in addition to the sensitization question, and that is, have you tried this procedure using autografts where rejection would not be a problem? I also am concerned about the gradual decline in function of these cells, which Dr. Orloff noted a few years ago when he presented his work regarding rat autografts.

DR. JAMES CERILLI (Rochester, New York): I would like to ask two simple questions. Have you done any work as to whether or not it is possible to revitalize this membrane as it gradually gets clogged? Can this be accomplished by an injection of any sort of material or living substances into the chambers?

And secondly is it possible to recharge these chambers *in vivo* so that if a patient lost function of the islets that you could, without removing the implant, recharge it by secondary injections of islets?

DR. EDWIN L. KAPLAN (Chicago, Illinois): Can you give us some idea of the number of islets that are used? Are the islets from one dog pancreas sufficient to correct the dog's sugar, for example?

DR. STANLEY R. FRIESEN (Kansas City, Kansas): Dr. Hardy has asked whether glucagon was present in the efferent limb of the pump. To explain the problem of weight loss in some of these dogs, it also might be important to know whether somatostatin or pancreatic polypeptide, especially, are released by the islet cells in this preparation.

DR. ANTHONY P. MONACO (Closing discussion): Thank you. I will answer the questions from the last to the first. Dr. Friesen, we have stored all the serum from these dogs to study output of glucagon and somatostatin and other hormones. We have found out that it is very difficult to find somebody who can do this for the canine allografts and for the xenografts, but we have the serum stored, and we plan to do this.

Dr. Cerilli asked about revitalization or reseeding of the devices. I left out the most important thing on the diagram, and that is that the device is constructed with two ports that look like small subcutaneous infusaports. The plan would be to place the device in an anatomic location where one could recharge it through these ports, that is, inject new islets into it, just by a needle through the skin. So reseeding is the most important concept in the device.

Dr. Corry mentioned the question of the gradual decline in the use of autografts. Gradual decline of islet function plagues the entire field. He mentions Dr. Orloff's experiments where rat autografts declined function. Dr. Gray and Dr. Morris transplanted autografts in monkeys intraportally, and over the 6 to 8 months, these autografts eventually failed and the animals became diabetic. So this is a problem probably in the device, but also even when autografts are transplanted and eventually make direct vascular anastomoses, and it is something we will all have to confront.

As far as clotting is concerned, Dr. Hardy, Dr. Barker, and Dr. Corry mentioned that. Clotting on the membrane has not been studied in great detail. I did not have a chance to show that there is a thin film of fibrin on the blood side of the membrane, but we have not done electron microscopy. Actually many of these membranes worked in such a way that the fibrin deposition is in fact necessary, because the pore size does not limit some of the lymphokines and cytokines *in vitro* from entrance, but the presence of fibrin actually makes a smaller physiologic pore and makes them work a little better. Conversely if too much fibrin is deposited, theoretically one would not get any nutrient flow. We have not investigated that.

As far as Dr. Barker's and Dr. Hardy's questions about the immune response, we have not studied it other than by histology in the devices. We plan to do immunoglobulin staining and electron microscopy, and we have the serum stored for antibody.

Diabetic complications in the long-term dogs have not been found, although a careful study to show a difference has not been done.

And then finally let me say it is my belief that we will transplant organs and cells, so-called naked, that is, without immunosuppression. This is one step that will be used in the next 10 or 20 years. The source, the possibility of transplanting cells, however, of any type, in vascular grafts is one of the most important new concepts that there is now. And in fact the genetically engineered cells that Dr. Barker talked about probably will be seeded in vascular grafts, so that they will be next to the bloodstream and be able to do whatever work we want them to do.

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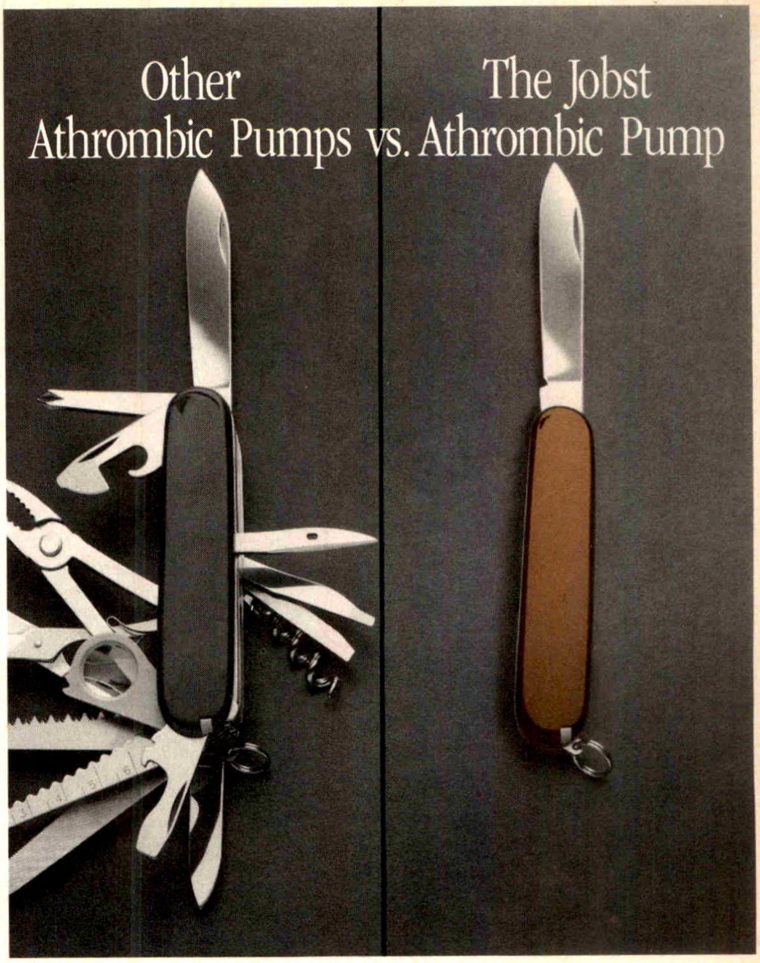

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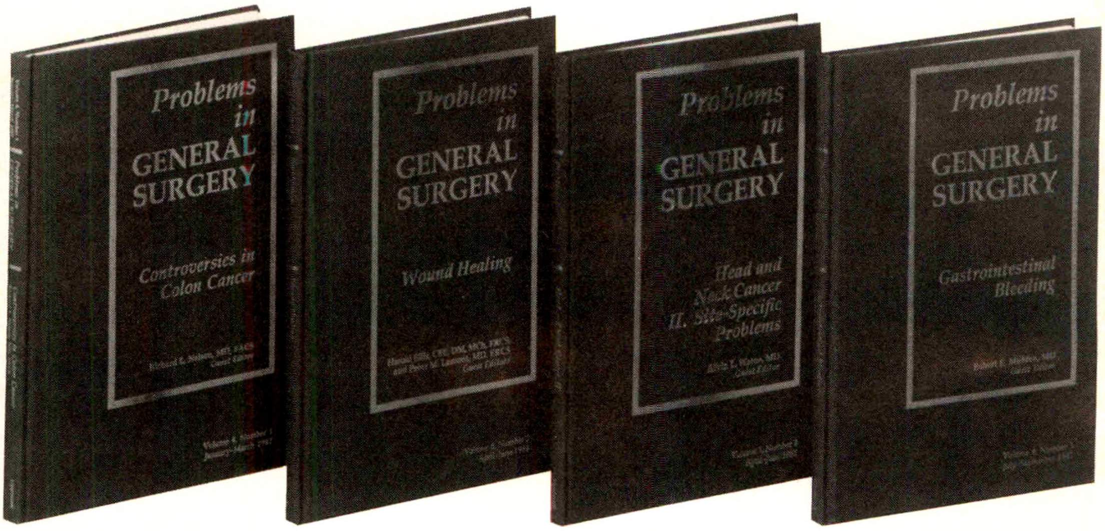
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November 2-3, 1991

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The course is intended for the physician, academician and researcher who uses liver biopsy in practice or clinical research and wants to update his or her knowledge of liver histopathology.

The purpose of the meeting will be to review important issues in liver biopsy interpretation with an emphasis on how liver biopsy can elucidate problems of differential diagnosis. The course is based upon syndromes and clinical presentations rather than specific diseases. The sessions will include discussions by clinicians and pathologists and will include clinical cases. A set of slides will accompany the syllabus which will be given to all registrants and sent out to preregistrants 1 month prior to the course.

On Saturday, November 2 there will be two sessions, one dealing with cholestatic syndromes and one with chronic hepatitis. On Sunday, November 3 the course will deal with liver disease after transplantation, evaluation of liver masses, cirrhosis and end stage liver disease and miscellaneous issues.

The course will be supplemented by the “Meet-the-Professor” luncheons. Course members will be able to attend these small, informal groups and interact with experts in a specific field.

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GENERAL SURGEONS

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